

Cortés, J. et al. Science (1991) 2523:675-679), which stated that the origins of the starter units for DEBS can include methylmalonate units which are loaded onto module 1 and are decarboxylated by the KS of module 1 (Pieper, R. et al. Biochemistry (1997) 36:1846-1851). It has now been found that when the DEBS1-TE protein is fully purified from extracts of recombinant *Sacch. erythraea* it contains no such specific decarboxylase activity (Weissmann, K. et al. (1998) Biochemistry, 37, 11012-11017), further confirming that starter units do not in fact arise from decarboxylation of extension units mediated by the KS of extension module 1 .

It is known that the DEBS loading module has a slightly broader specificity than propionate only, and in particular acetate starter units are used both in vitro and in vivo, when the PKS containing this loading module is part of a PKS that is expressed either in *Sacch. erythraea* the natural host for erythromycin production (see for example Cortés, J. et al. Science (1995) 268:1487-1489), or in an heterologous host such as *S. coelicolor* (Kao, C. M. et al. J. Am. Chem. Soc. (1994) 116:11612-11613; Brown, M. J. B. et al. J. Chem. Soc. Chem. Commun. (1995) 1517-1519). In vitro experiments using purified DEBS1-TE have demonstrated that propionyl-CoA and acetyl-CoA are alternative substrates that efficiently supply propionate and acetate units respectively to the loading module (Wiessmann, K. E. H. et al. Chemistry and Biology (1995)

2:583-589; Pieper, R. et al. J. Am. Chem. Soc. (1995)
117:11373-11374). The outcome of the competition between
acetate and propionate starter units is influenced by the
respective intracellular concentrations of propionyl-CoA
5 and acetyl-CoA prevailing in the host cell used (see for
example Kao, C. M. et al. Science (1994) 265:509-512;
Pereda, A. et al. Microbiology (1995) 144:543-553). It is
also determined by the level of expression of the host PKS,
so that as disclosed for example in Pending International
10 Patent Application number PCT/GB97/01819, when recombinant
DEBS or another hybrid PKS containing the DEBS loading
module is over-expressed in *Sacch. erythraea*, the products
are generally mixtures whose components differ only in the
presence of either an acetate or a propionate starter unit.

15 There is a need to develop reliable methods for
avoiding the formation of mixtures of polyketides with both
acetate and propionate starter units, and to allow the
specific incorporation of unusual starter units. It has now
20 been found, surprisingly, that the role of the loading
domains in the PKSs for the 16-membered macrolides tylosin,
niddamycin and spiramycin is different from that of the
loading domains of the avermectin PKS and of DEBS. It has
been realised that the KSq domain of the tylosin PKS and
25 the associated AT domain, which is named here ATq, together
are responsible for the highly specific production of
propionate starter units because the ATq is specific for
the loading of methylmalonyl-CoA and not propionyl-CoA as
previously thought; and the KSq is responsible for the

highly specific decarboxylation of the enzyme-bound methylmalonate unit to form propionate unit attached to the ACP domain of the loading module and appropriately placed to be transferred to the KS of extension module 1 for the initiation of chain extension. In a like manner the ATq of the spiramycin and niddamycin PKSs, and the adjacent KSq, are responsible for the specific loading of malonate units rather than acetate units as previously believed, and for their subsequent specific decarboxylation to provide acetate starter units for polyketide chain extension.

It has also now been found here that not only the PKSs for the above-mentioned 16-membered macrolides, but also the PKSs for certain 14-membered macrolides particularly the oleandomycin PKS from *Streptomyces antibioticus* (Figure 4) and also the PKSs for certain polyether ionophore polyketides particularly the putative monensin PKS from *Streptomyces cinnamonensis* (Figure 4), possess a loading domain comprising a KSq domain, an ATq domain, and an ACP. In Figure 4 is shown a sequence alignment of the KSq domains and of the adjacent linked ATq domains that have been identified, showing the conserved active site glutamine (Q) residue in the KSq domains, and an arginine residue which is conserved in all extension AT domains and is also completely conserved in ATq domains. This residue is characteristically not arginine in the AT domains of either DEBS or of the avermectin PKS loading modules, where the substrate for the AT is a non-carboxylated acyl-CoA ester (Haydock, S. F. et al. FEBS Letters (1995) 374:246-248) . The abbreviation ATq is used here to simply to

distinguish the AT domains found immediately C-terminal of Ksq from extension ATs, and the label has no other significance.

In one aspect this invention provides a PKS
5 multienzyme or part thereof, or nucleic acid (generally DNA) encoding it, said multienzyme or part comprising a loading module and a plurality of extension modules for the generation of novel, 14-membered macrolides wherein

(a) the loading module is adapted to load a malonyl
10 residue and then to effect decarboxylation of the loaded residue to provide an acetyl residue for transfer to an extension module; and

(b) the extension modules, or at least one thereof
15 (preferably at least the one adjacent the loading module), are not naturally associated with a loading module that effects decarboxylation of an optionally substituted malonyl residue.

Generally the loading module will also include an ACP (acyl carrier protein) domain.

20 Preferably the decarboxylating functionality of the loading module is provided by a KS (ketosynthase)-type domain. Suitably this differs from a KS of a conventional extension module by possessing a glutamine residue in place of the essential cysteine residue in the active site. It
25 is termed Ksq. It may be "natural" or genetically engineered, e.g. resulting from site-directed mutagenesis of nucleic acid encoding a different KS such as a KS of an extension module.

Alternatively the decarboxylating functionality can be provided by a CLF-type domain of the general type occurring in Type II PKS systems.

Preferably the loading functionality is provided by an AT (acyltransferase)-type domain which resembles an AT domain of a conventional extension module in having an arginine residue in the active site, which is not the case with the AT domains of loader modules which load acetate or propionate, e.g. in DEBS or avermectin PKS systems. It may be termed Atq. Once again, it may be "natural" or genetically engineered, e.g. by mutagenesis of an AT of an extension module.

Usually the loading module will be of the form:

Ksq-ATq-ACP

where ACP is acyl carrier protein.

In another aspect the invention provides a method of synthesising novel, 14-membered polyketides having substantially exclusively a desired acetate starter unit by providing a PKS multienzyme incorporating a loading module as defined above which specifically provides the desired acetate starter unit. This may comprise providing nucleic acid encoding the multienzyme and introducing it into an organism where it can be expressed.

In further aspects the invention provides vectors and transformant organisms and cultures containing nucleic acid encoding the multienzyme. A preferred embodiment is a culture which produces a 14-membered polyketide having a desired acetate starter unit characterised by the substantial absence of polyketides with different starter units. Thus, for example, C13-methyl-erythromycin can be

produced substantially free from natural analogues resulting from the incorporation of propionate starter units.

It is particularly useful to provide a loading module of the type KSq - ATq-ACP for a PKS gene assembly which produces a 14-membered macrolide in order to prepare a 14-membered macrolide which contains exclusively or almost exclusively an acetate starter unit, even when such PKS gene assembly is expressed at high levels in an actinomycete host cell. Particularly suitable PKSs for this purpose are the components of PKSs for the biosynthesis of erythromycin, methymycin, oleandomycin, tylosin, spiramycin, midecamycin, and niddamycin for all of which the gene and modular organisation is known at least in part. Particularly suitable sources of the genes encoding a loading module of the type KSq - ATq-ACP are the loading modules of oleandomycin, spiramycin, niddamycin, methymycin and monensin which are specific for the loading of malonate units which are then decarboxylated to acetate starter units.

In the loading module of the type KSq - ATq-ACP the domains or portions of them may be derived from the same or from different sources, and comprise either natural or engineered domains. For example the ATq domain can be replaced by an AT domain derived from any extension module of a Type I PKS, having specificity for loading of malonate units, so long as the KSq domain is chosen to have a matching specificity towards malonate units.

Alternatively, the KSq domain in the loading module provided of the type KSq - ATq-ACP may be substituted by

the CLF polypeptide of a Type II PKS. It is now apparent that in contrast to its previous identification as a factor uniquely determining chain length, the CLF, in addition to any other activities that it may possess, is the analogue of the KSq domain and can act as a decarboxylase towards bound malonate units.

The appreciation that the CLF domain of Type II PKS's has decarboxylating activity has led us to devise useful interventions in Type II systems, e.g. to enhance the yields obtainable in some fermentations. Many high-yielding industrial fermentations tend to give mixtures, owing to the incorporation of undesired starters. This is particularly the case in systems which have auxiliary genes for generating unusual starters. CLF genes may act to produce undesired acyl species, leading to products incorporating the undesired acyl units.

For example the production of oxytetracycline involves an unusual malonamido starter. However the undesired activity of a CLF domain causes some decarboxylation, leading to the incorporation of acetyl instead. Daunomycin synthesis likewise involves an unusual starter which is liable to the "parasitic" activity of a CLF domain.

The active site (for decarboxylation) of a CLF domain generally includes a glutamine residue. We find that the decarboxylating activity of the domain can be removed by a mutation by which the Gln residue is converted into (for example) Ala.

Thus in a further aspect the invention provides a

system and process for synthesis of a type II (aromatic) polyketide, in which a gln residue of a CLF domain of the type II PKS is mutated to suppress decarboxylation activity. Techniques of site-specific mutagenesis by which this can be achieved are by now well known to those skilled in the art.

The loading module of the type KSq - ATq-ACP may be linked to a hybrid PKS produced for example as in PCT/GB97/01819 and PCT/GB97/01810. It is particularly useful to link such a loading module to gene assemblies that encode hybrid PKSs that produce novel derivatives of 14-membered macrolides as described for example in PCT/GB97/01819 and PCT/GB97/01810.

The invention further provides such PKS assemblies furnished with a loading module of the type KSq - ATq-ACP, vectors containing such assemblies, and transformant organisms that can express them. Transformant organisms may harbour recombinant plasmids, or the plasmids may integrate. A plasmid with an *int* sequence will integrate into a specific attachment site (*att*) of the host's chromosome. Transformant organisms may be capable of modifying the initial products, eg by carrying out all or some of the biosynthetic modifications normal in the production of erythromycins (as shown in Figure 5) and for other polyketides. Use may be made of mutant organisms such that some of the normal pathways are blocked, e.g. to produce products without one or more "natural" hydroxy-groups or sugar groups. The invention further provides novel polyketides as producible, directly or indirectly, by

transformant organisms. This includes polyketides which have undergone enzymatic modification.

In a further aspect the invention provides both previously-obtained 14-membered ring macrolides and novel 14-membered ring macrolides in a purer form with respect to the nature of the acetate starter unit, than was hitherto possible. These include 14-membered ring macrolides which are either "natural" or may differ from the corresponding "natural" compound:

- a) in the oxidation state of one or more of the ketide units (ie selection of alternatives from the group: -CO-, -CH(OH)-, alkene -CH-, and -CH₂-) where the stereochemistry of any -CH(OH)- is also independently selectable;
- b) in the absence of a "natural" methyl side-chain; or
- c) in the stereochemistry of "natural" methyl; and/or ring substituents other than methyl.

It is also possible to prepare derivatives of 14-membered ring macrolides having the differences from the natural product identified in two or more of items a) to c) above.

Derivatives of any of the afore-mentioned polyketides which have undergone further processing by non-PKS enzymes, eg one or more of hydroxylation, epoxidation, glycosylation and methylation may also be prepared.

The present invention provides a novel method of obtaining both known and novel complex 14-membered macrolides having an acetate starter unit substantively

free of products differing only in having a propionate starter unit.

Suitable plasmid vectors and genetically engineered cells suitable for expression of PKS genes incorporating an altered loading module are those described in PCT/GB97/01819 as being suitable for expression of hybrid PKS genes of Type I. Examples of effective hosts are *Saccharopolyspora erythraea*, *Streptomyces coelicolor*, *Streptomyces avermitilis*, *Streptomyces griseofuscus*, *Streptomyces cinnamonensis*, *Streptomyces fradiae*, *Streptomyces longisporoflavus*, *Streptomyces hygroscopicus*, *Micromonospora griseorubida*, *Streptomyces lasaliensis*, *Streptomyces venezuelae*, *Streptomyces antibioticus*, *Streptomyces lividans*, *Streptomyces rimosus*, *Streptomyces albus*, *Amycolatopsis mediterranei*, and *Streptomyces tsukubaensis*. These include hosts in which SCP2*-derived plasmids are known to replicate autonomously, such as for example *S. coelicolor*, *S. avermitilis* and *S. griseofuscus*; and other hosts such as *Saccharopolyspora erythraea* in which SCP2*-derived plasmids become integrated into the chromosome through homologous recombination between sequences on the plasmid insert and on the chromosome; and all such vectors which are integratively transformed by suicide plasmid vectors.

Although some 13-methyl erythromycins (also known as 15-norerythromycins) have been reported previously (Kibwage et

al., J. Antibiotics, 40, 1-6, 1987; Weber & McAlpine, U.S. Patent 5,141,926), these have been confined to 15-norerythromycin C, and 6-deoxy-15-norerythromycins B and D. Moreover, not only have these 15-norerythromycins been found as extremely minor components co-expressed with high levels of "natural" erythromycins (13-ethyl erythromycins), but the 13-methyl counterparts (15-norerythromycins A and B) to the most desirable and biologically-active "natural" erythromycins (erythromycin A and B) have never been previously isolated. Chemical modification of "natural" erythromycins has proven to be an extremely effective means for enhancing the bioefficacy of the "natural" molecules. Thus, it would be envisaged that chemical modification of novel erythromycins would similarly produce compounds with desirable and enhanced bioefficacies. PCT/GB97/01819 describes in general terms the production of novel polyketides through recombinant DNA technologies, and the use of these technologies to generate novel erythromycins, many of which have different starter units to the propionate starter unit characteristic of the "natural" erythromycins, are described in pending International Patent Application PCT/GB97/01810, Some chemical modification of these novel erythromycins are also described in co-pending International Patent Applications PCT/IB98/02100 and PCT/IB98/02099. However, it is clear that the ability to produce novel erythromycins at good expression levels and in the substantial absence of novel or natural

erythromycins with different starter units is essential to facilitate the ability to achieve a wide range of chemical modifications to such novel erythromycins. The enhanced ability to produce polyketides at good expression levels and in the substantial absence of polyketides with different starter units has been described in this application are family members, and we now describe the ability to produce 13-methyl erythromycins at good expression levels and in the substantial absence of erythromycins with different starter units. The use of this technology has now permitted the preparation of large amounts of 13-methyl erythromycins which for the first time has permitted us to carry out a wide range of chemical modifications which had only been previously possible starting from the "natural" erythromycins.

Some embodiments of the invention will now be described with reference to the accompanying drawings in which:

Fig 1 is a diagram showing the functioning of 6-deoxyerythronolide B synthase (DEBS), a modular PKS producing 6-deoxyerythronolide B (6-DEB) a precursor of erythromycin A.

Fig 2 gives the amino acid sequence comparison of the KS domains and the CLF domains of representative Type II PKS gene clusters. The active site Cysteine (C) of the KS domains is arrowed in the Figure and aligns with the Glutamine (Q) or glutamic acid (E) of the CLF domains. The

abbreviations used, and the relevant Genbank/EMBL accession numbers are: GRA: granaticin from *Streptomyces violaceoruber* (X63449); HIR: unknown polyketide from *Saccharopolyspora hirsuta* (M98258); ACT, actinorhodin from *Streptomyces coelicolor* (X63449); CIN: unknown polyketide from *Streptomyces cinnamonensis* (Z11511); VNZ: jadomycin from *Streptomyces venezuelae* (L33245); NOG: anthracyclines from *Streptomyces nogalater* (Z48262); TCM: tetracenomycin from *S. glaucescens* (M80674); DAU: daunomycin from *Streptomyces sp. C5* (L34880); PEU, doxorubicin from *Streptomyces peucetius* (L35560); WHI: WhiE spore pigment from *Streptomyces coelicolor* (X55942).

Fig 3 shows the gene organisation of the PKSs for three 16-membered ring macrolides, tylosin, spiramycin and niddamycin.

Fig 4 shows the amino acid sequence alignment of KSq-ATq loading didomains of the PKSs for niddamycin, platenolide(spiramycin), monensin, oleandomycin and tylosin. The sequences for the monensin and oleandomycin loading didomains have not been previously disclosed.

Fig. 5 The enzymatic steps that convert 6-deoxyerythronolide B into erythromycin A in *Saccharopolyspora erythraea*

Fig. 6 is a diagram showing the construction of plasmid pJLK117.

Fig. 7 shows the structures of two oligonucleotides.

The present invention will now be illustrated, but is

not intended to be limited, by means of some examples.

All NMR spectra were measured in CDCl₃ using a Bruker 500MHz DMX spectrometer unless otherwise indicated and peak positions are expressed in parts per million (ppm) downfield from tetramethylsilane. The atom number shown in the NMR structure is not representative of standard nomenclature, but correlates NMR data to that particular example.

HPLC methods

Method A

Column Waters Symmetry 5_ C18 2.1mm X 150 mm
 Flow 0.29 ml/min
 Mobile phase Gradient: A:B (22:78) to A:B (38:62) over 12 minutes, then to A:B (80:20) by minute 15. Maintain for 1 minute. Re-equilibrate before next sample. Where A = acetonitrile and B = 0.01M ammonium acetate in 10% acetonitrile and 0.02% TFA
 Instrument Acquired with Hewlett-Packard 1050 liquid chromatograph interfaced to a VG Platform II mass spectrometer equipped with an APCI source

Method B

Column Waters Symmetry 5_ C18 2.1mm X 150 mm
 Flow 0.29 ml/min
 Mobile phase Gradient: 28:72 acetonitrile:10mM NH₄OAc to 50:50 in 18 minutes. 50:50 until 25 minutes. Back to 28:72, re-equilibrate for 7 minutes
 Instrument Acquired with Hewlett Packard 1100 LC/MS with APCI source

Tap Water medium

glucose 5g/liter
 tryptone 5g/liter
 yeast extract 2.5g/liter
 EDTA 36mg/liter
 Tap water to 1L total volume

ERY - P medium

dextrose 50g/liter
 NutrisoyTM flour 30g/liter
 (NH₄)₂SO₄ 3g/liter
 NaCl 5g/liter
 CaCO₃ 6g/liter
 Tap water to 1L total volume
 pH adjusted to 7.0

Example 1

Construction of the Recombinant Vector pPFL43

5 Plasmid pCJR24 was prepared as described in PCT/GB97/01819. pPFL43 is a pCJR24-based plasmid containing the gene encoding a hybrid polyketide synthase that contains the putative monensin PKS loading module (isolated from *S. cinnamomensis*) the DEBS extension
10 modules 1 and 2 and the chain-terminating thioesterase. Plasmid pPFL43 was constructed as follows:

The following synthetic oligonucleotides: 5'-

CCATATGGCCGCATCCGCGTCAGCGT-3' and 5'-

15 GGCTAGCGGGTCCTCGTCCGTGCCGAGGTCA-3'

are used to amplify the DNA encoding the putative monensin-producing loading module using a cosmid that contains the 5' end of the putative monensin-producing PKS genes from *S. cinnamomensis* or chromosomal DNA of *S.*
20 *cinnamomensis* as template. The PCR product of 3.3 kbp is purified by gel electrophoresis, treated with T4 polynucleotide kinase and ligated to plasmid pUC18, which has been linearised by digestion with *Sma* I and then treated with alkaline phosphatase. The ligation mixture
25 was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid pPFL40. Plasmid pPFL40 was identified by restriction pattern and sequence analysis.

Plasmid pHD30His is a derivative of pNEWAVETE (PCT/GB97/01810) which contains the avermectin loading module, erythromycin extension modules 1 and 2 and the ery thioesterase domain. Plasmid pNEWAVETE was cut with
5 EcoRI and HindIII and a synthetic oligonucleotide linker was inserted that encodes the addition of a C-terminal polyhistidine tail to the polypeptide. The following oligonucleotides:

5'-AATTCACATCACCATCACCATCACTAGTAGGAGGTCTGGCCATCTAGA-3'

10 and

5'-AGCTTCTAGATGGCCAGACCTCCTACTAGTGATGGTGATGGTGATGTG-3'

were annealed together and the duplex was ligated to EcoRI-and HindIII-cut pNEWAVETE. The resulting plasmid was cut with NdeI and XbaI and ligated into plasmid
15 pCJR24 that had been previously cut with same two enzymes, to produce plasmid pND30His.

Plasmid pPFL40 was digested with *Nde* I and *Nhe* I and the 3.3 kbp fragment was purified by gel electrophoresis and ligated to pND30-His previously digested with *Nde* I
20 and *Nhe* I and treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid pPFL43. Plasmid pPFL43 was identified by restriction analysis.

Example 2

Construction of *S. erythraea* NRRL2338/pPFL43

Plasmid pPFL43 was used to transform *S. erythraea* NRRL2338 protoplasts. Thiostrepton resistant colonies were selected in R2T20 medium containing 10 µg/ml of thiostrepton. Several clones were tested for the presence of pPFL43 integrated into the chromosome by Southern blot hybridisation of their genomic DNA with DIG-labelled DNA containing the *mon* PKS fragment encoding for the loading module. A clone with an integrated copy of pPFL43 was selected in this way.

Example 3

Production of 13-methyl-erythromycin A and B using *Sacch. erythraea* NRRL 2338/pPFL43

The culture *Saccharopolyspora erythraea* NRRL2338(pPFL43), constructed with the wild-type loading domain displaced by a monensin loader-D1TE DNA insert, produced as described in Example 2, was inoculated into 30ml Tap Water medium with 50 µg/ml thiostrepton in a 300ml Erlenmeyer flask. After three days incubation at 29°C, this flask was used to inoculate 300 ml of ERY-P medium in a 300 ml flask. The broth was incubated at 29°C at 200 rpm for 6 days. After this time, the whole broth was adjusted to pH 8.5 with NaOH, then extracted with equal volume of ethyl acetate. The ethyl acetate extract was evaporated to dryness at 45°C under a nitrogen stream using a Zymark TurboVap LV Evaporator, then reconstituted in 0.0625 volumes methanol to concentrate the extract 16-fold. The structures of the products were confirmed by LC/MS, Method A. A 4.0 min retention time peak was observed as the major component, with *m/z* value of 720 (M+H)⁺, required for 13-methyl-erythromycin A. A second peak was observed with a retention time of 6.4 min and with *m/z* value of 704 (M+H)⁺, required for 13-methyl-erythromycin B.

Example 4

Production and Recovery of 13-methyl-erythromycin A and B using *Sacch. erythraea* NRRL-2338 (pPFL43) at 8L scale

Saccharopolyspora erythraea NRRL2338 (pPFL43) was inoculated into 1000mls Tap Water medium with 50 µg/ml

thiostrepton in a 2.8l Fernbach flask. After three days incubation at 29°C, this flask was used to inoculate 8l of ERY-P medium in a 14l Microferm fermentor jar (New Brunswick Scientific Co., Inc., Edison, NJ). The broth was incubated at 28°C with an aeration rate of 8l/min, stirring at 800 rpm and with pH maintained between 6.9 and 7.3 with NaOH or H₂SO₄ (15%). Water was added to maintain volume at the 24 hour volume level. The fermentation was continued for 167 hours. After this time, presence of 13-methyl-erythromycin A and B were confirmed by adjusting a broth sample from the fermentor to pH 8.5 with NaOH, then extracting with equal volume of ethyl acetate. The ethyl acetate extract was evaporated to dryness at 45°C under a nitrogen stream using a Zymark TurboVap LV Evaporator, then reconstituted in 0.25 volumes methanol to concentrate the extract 4-fold. The structures of the products were confirmed by LC/MS, Method A. A 4.1 min retention time peak was observed as the major component, with *m/z* value of 720 (M+H)⁺, required for 13-methyl-erythromycin A. A second peak was observed with a retention time of 6.6 min and with *m/z* value of 704 (M+H)⁺, required for 13-methyl-erythromycin B.

About 35 liters of broth containing approximately 2.8 grams of 13-methyl-erythromycin A were processed for recovery of product. Broth was filtered through a pilot sized Ceraflo ceramic unit and loaded onto a 500ml XAD-16 resin column. The product was eluted using 100% methanol. A 175ml CG-161 adsorption column was prepared and equilibrated with 20% methanol/water. A portion of the product solution was adjusted to 20% methanol and loaded onto the column, no breakthrough of product was observed. Washing of the column with up to 40% methanol/water failed at removing any significant level of impurities. Elution with 50% methanol/water achieved chromatographic separation of the product from the two major impurities, 13-methyl-erythromycin B and a degradation product, 13-methyl-dehydroerythromycin A. The purest cuts were combined and reduced in volume by approximately 75% using evaporation to achieve <10% methanol concentration. To enhance 13-methyl-erythromycin A extraction, solid sodium bicarbonate was added until a total concentration of 250mM was obtained. The aqueous product layer was extracted 2x with methylene chloride, using one-half the total volume each time. The volume was reduced to light yellow solids by evaporation. The 13-methyl-erythromycin A was purified by dissolving the crude crystals into methylene chloride at ambient temperature and diluting to 15% methylene chloride with hexane. The cloudy solution is placed at -10°C for ~30 minutes when the liquid is decanted to a 2nd flask, leaving the majority of impurities behind as an oil. The flask is left overnight at -10°C, followed by filtration of off-white 13-methyl-erythromycin A crystals the next day. Approximately 300 milligrams of 13-methyl-erythromycin A were isolated from the partial work-up of the 35l broth volume.

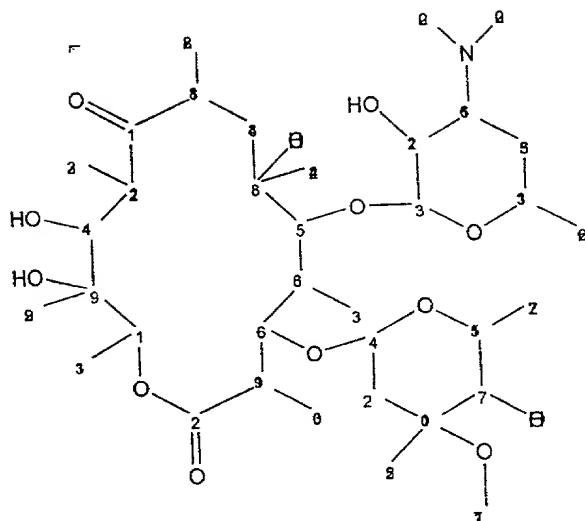
Approximately 100 grams of evaporated mother liquor were utilized further to isolate 13-methyl-erythromycin B. Residual 13-methyl-erythromycin A was removed with repetitive extraction of the initial sample with aqueous acetic acid (pH 5). The subsequent methylene chloride layer was chromatographed on 700 g of silica gel using 20% methanol in methylene chloride. The 13-methyl-erythromycin B enriched fractions, as determined by LC/MS, were combined and evaporated to yield ~11.0 grams of dark oil. The oil was dissolved in a minimal amount of methanol and loaded onto 500 ml of Amberchrom CG-161 resin. The 13-methyl-erythromycin B was eluted at 2 bed volumes per hour with 40% methanol in deionized water. One bed volume fractions were collected and assayed by LC/MS. Fractions 42 through 62 were combined, diluted to ~20% methanol with deionized water, and neutralized to pH 7.5 with sodium bicarbonate. The resulting solution was extracted once with 4l of methylene chloride, concentrated to ~500 ml, and dried over anhydrous magnesium

sulfate. After removal of the MgSO_4 by filtration the filtrate was evaporated to give ~110 mg of light brown solids. The 110 mg of crude 13-methyl-erythromycin B was dissolved in ~ 3.0 milliliters of HPLC grade acetonitrile and loaded onto a 20cm x 20cm, 2mm thick, silica gel preparative thin layer chromatography (PTLC) plate. The plate was developed with 60:40 methanol:acetonitrile. The desired portion of silica from the PTLC plate (iodine visualisation) was removed and extracted with HPLC grade acetone. The acetone extract was evaporated to give 12.1 mg of clear solid.

Identification of the 13-methyl-erythromycin A and 13-methyl-erythromycin B samples were confirmed by mass spectroscopy (LC/MS Method B) and NMR spectroscopy. The 13-methyl-erythromycin A sample peak had a 4.7 min retention time, with m/z value of 720 $(\text{M}+\text{H})^+$, required for 13-methyl-erythromycin A. The 13-methyl-erythromycin B sample peak had a 7.6 min retention time, with m/z value of 704 $(\text{M}+\text{H})^+$, required for 13-methyl-erythromycin B.

NMR, 13-methyl-erythromycin A:

31



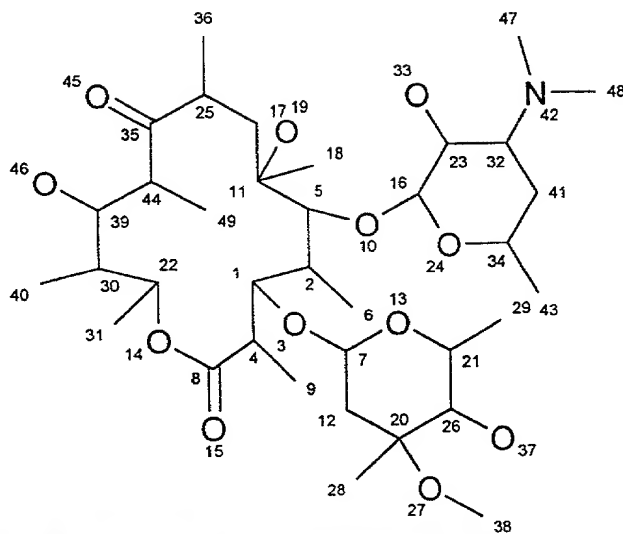
T06780-74902659

	#	¹³ C - ppm	#H	¹ H - ppm
5	1	221.91	0	
	2	175.99	0	
	3	103.63	1	4.45
10	4	96.81	1	4.88
	5	83.76	1	3.60
	6	79.86	1	4.10
	7	78.36	1	3.05
	8	75.50	0	
15	9	74.87	0	
	10	73.07	0	
	11	72.25	1	5.19
	12	71.25	1	3.26
	13	69.53	1	3.53
20	14	69.24	1	3.97
	15	66.16	1	4.06
	16	65.96	1	2.48
	17	49.96	3	3.36
	18	45.36	1	2.79
	19	45.07	1	2.81
25	20	40.73	3	2.32
	21	39.00	1	3.15
	22	35.30	2	2.42/1.61
	24	27.20	3	1.50
30	25	21.92	3	1.28
	26	21.82	3	1.27
	27	18.99	3	1.32
	28	18.60	3	1.22
	29	16.07	3	1.19
35	30	15.08	3	1.19
	31	14.23	3	1.26
	32	12.12	3	1.19

33	9.60	3	1.15
34	39.00	2	1.98/1.75
35	28.90	2	1.72/1.27
36	40.94	1	2.05

5

NMR, 13-methyl-erythromycin B:



10

15

20

25

#	13C - PPM	#H attached	1H - PPM
1	80.50	1	4.15
2	40.62	1	2.15
4	45.17	1	2.84
5	84.08	1	3.62
6	9.86	3	1.18
7	97.26	1	4.88
8	176.48	0	
9	15.25	3	1.22
11	75.98	0	
12	35.43	2	2.42/1.61
16	103.75	1	4.46
17	38.77	2	2.09/1.72
18	27.67	3	1.51
20	73.09	0	
21	66.20	1	4.06
22	70.27	1	5.58
23	71.24	1	3.28
25	45.49	1	2.81

	26	78.29	1	3.06
	28	21.91	3	1.28
	29	19.03	3	1.33
	30	41.61	1	1.65
5	31	18.73	3	1.29
	32	65.94	1	2.53
	34	69.52	1	3.55
	35	219.92	0	
	36	19.03	3	1.21
10	38	49.97	3	3.36
	39	70.17	1	3.88
	40	9.27	3	0.95
	41	29.12	2	1.73/1.28
	43	21.80	3	1.27
15	44	39.87	1	3.07
	47	40.74	3	2.35
	48	40.74	3	2.35
20	49	9.62	3	1.04

TABLE 4

Example 5Construction of plasmid pPFL35

Plasmid pPFL35 is a pCJR24-based plasmid containing a PKS gene comprising a loading module, the first and second extension modules of DEBS and the chain terminating thioesterase. The loading module comprises the KSq domain DNA from the loading module of the oleandomycin PKS fused to the malonyl-CoA-specific AT of module 2 of the rapamycin PKS, in turn linked to the DEBS loading domain ACP. Plasmid pPFL35 was constructed via several intermediate plasmids as follows:

A 411 bp DNA segment of the *eryAI* gene from *S. erythraea* extending from nucleotide 1279 to nucleotide 1690 (Donadio, S. et al., Science (1991) 2523:675-679) was amplified by PCR using the following synthetic oligonucleotide primers:-

5'-TGGACCGCCGCCAATTGCCTAGGCGGGCCGAACCCGGCT-3' and

5'-CCTGCAGGCCATCGCGACGACCGCGACCGGTTCCGCC-3'

The DNA from a plasmid designated pKSW, derived from pT7-7 and DEBS1-TE in which new *Pst* I and *Hind*III sites had been introduced to flank the KS1 of the first extension module, was used as a template. The 441 bp PCR product was treated with T4 polynucleotide kinase and ligated to plasmid pUC18, which had been linearised by digestion with *Sma* I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E. coli* DH10B cells and individual clones were checked for the desired plasmid, pPFL26. The new *Mfe* I/*Avr* II sites

bordering the insert are adjacent to the *Eco* RI site in the polylinker of pUC18. Plasmid pPFL26 was identified by restriction pattern and sequence analysis.

An *Mfe* I restriction site is located 112 bp from the 5' end of the DNA encoding the propionyl-CoA:ACP transferase of the loading module of DEBS. Plasmid pKSW was digested with *Mfe* I and *Pst* I and ligated with the 411 bp insert obtained by digesting plasmid pPFL26 with *Mfe* I and *Pst* I. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid, pPFL27. Plasmid pPFL27 contains a PKS gene comprising the DEBS loading module, the first and second extension modules of DEBS and the DEBS chain terminating thioesterase. Plasmid pPFL27 was identified by its restriction pattern.

Plasmid pPFL27 was digested with *Nde* I and *Avr* II and ligated to a 4.6kbp insert derived from digesting plasmid pMO6 (PCT/GB97/01819) with *Nde* I and *Avr* II. Plasmid pMO6 contains a PKS gene comprising the DEBS loading module, the first and second extension modules of DEBS and the DEBS chain terminating thioesterase, except that the DNA segment encoding the methylmalonate-specific AT within the first extension module has been specifically substituted by the DNA encoding the malonate-specific AT of module 2 of the *rap* PKS. The ligation mixture was used to transform electrocompetent *E. coli* DH10B cells and individual clones were checked for the desired plasmid, pPFL28. Plasmid pPFL28 contains a hybrid PKS gene comprising the DEBS

loading module, the malonate-specific AT of module 2 of the rap PKS, the ACP of the DEBS loading module, followed by the first and second extension modules of DEBS and the DEBS chain terminating thioesterase. Plasmid pPFL28 was identified by restriction analysis.

A DNA segment encoding the KSq domain from the *oleAI* gene of *S. antibioticus* extending from nucleotide 1671 to nucleotide 3385 was amplified by PCR using the following synthetic oligonucleotide primers:-

10 5'-CCACATATGCATGTCCCCGGCGAGGAA-3' and
5'-CCCTGTCCGGAGAAGAGGAAGGCGAGGCCG-3'

and chromosomal DNA from *Streptomyces antibioticus* as a template. The PCR product was treated with T4 polynucleotide kinase and ligated to plasmid pUC18, which had been linearised by digestion with *Sma* I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E. coli* DH10B cells and individual clones were checked for the desired plasmid, pPFL31. The new *Nde* I site bordering the insert is adjacent to the *Eco* RI site of the pUC18 polylinker while the new *Bsp* EI site borders the *Hin* dIII site of the linker region. Plasmid pPFL31 was identified by restriction and sequence analysis.

Plasmid pPFL31 was digested with *Nde* I and *Avr* II and the insert was ligated with plasmid pPFL28 that had been digested with *Nde* I and *Avr* II. The ligation mixture was used to transform electrocompetent *E. coli* DH10B cells and

individual clones were checked for the desired plasmid, pPFL32. Plasmid pPFL32 was identified by restriction analysis.

Plasmid pPFL32 was digested with *Nde* I and *Xba* I and the insert was ligated to plasmid pCJR24, which had been digested with *Nde* I and *Xba* I and purified by gel electrophoresis. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid, pPFL35. Plasmid pPFL35 was identified by restriction analysis.

Example 6

Construction of *S. erythraea* NRRL2338/pPFL35

Plasmid pPFL35 was used to transform *S.erythraea* NRRL2338 protoplasts. Thiostrepton resistant colonies were selected in R2T20 medium (Yamamoto et al.) containing 10 µg/ml of thiostrepton. Several clones were tested for the presence of pPFL35 integrated into the chromosome by Southern blot hybridisation of their genomic DNA with DIG-labelled DNA containing the rap PKS fragment encoding for module 2 AT. A clone with an integrated copy of pPFL35 was identified in this way.

Example 7

Production of 13-methyl-erythromycin A and B using *Sacch. erythraea* NRRL-2338 (pPFL35)

The culture *Saccharopolyspora erythraea* NRRL2338(pPFL35), constructed with the wild-type loading

5

20

Construction of Recombinant Vector pPFL44

25

The following synthetic oligonucleotides:

30

5' - GGCTAGCGGGTCGTGTCGTCCCGGCTG - 3'

35

plasmid pUC18, which had been linearised by digestion with *Sma* I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid pPFL41. Plasmid pPFL41 was identified by restriction pattern and sequence analysis.

Plasmid pPFL41 was digested with *Nde* I and *Nhe* I and the 3.3 kbp fragment was purified by gel electrophoresis and ligated to pND30 (a plasmid derived from plasmid pCJR24 having as insert the ave PKS loading module and extension modules 1 and 2 or DEBS and the DEBS thioesterase) (PCTGB97/01810) previously digested with *Nde* I and *Nhe* I and treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones checked for the desired plasmid pPFL44. Plasmid pPFL44 was identified by restriction analysis.

20

Example 9

Construction of *Sacch. erythraea* NRRL2338/pPFL44

Plasmid pPFL44 was used to transform *S.erythraea* NRRL2338 protoplasts. Thiostrepton resistant colonies were selected in R2T20 medium containing 10 µg/ml of thiostrepton. Several clones were tested for the presence of pPFL44 integrated into the chromosome by Southern blot hybridisation of their genomic DNA with DIG-labelled DNA

containing the spiramycin PKS fragment encoding for the loading module. A clone with an integrated copy of pPFL44 was identified in this way.

5 **Example 10**

Production of 13-methyl-erythromycin A and B using *Sacch. erythraea* NRRL-2338 (pPFL44)

6972641-094301
T04290-1492269

10 The culture *Saccharopolyspora erythraea* NRRL2338 (pPFL44), constructed with the wild-type loading domain displaced by spiramycin loader-D1TE DNA insert, was inoculated into 30ml Tap Water medium with 50 ug/ml thiostrepton in a 300ml Erlenmeyer flask. After three days incubation at 29°C, this flask was used to inoculate 300 ml of ERY-P medium in a 300 ml flask. The broth was incubated at 29°C at 200 rpm for 6 days. After this time, the whole broth was adjusted to pH 8.5 with NaOH, then extracted with equal volume of ethyl acetate. The ethyl acetate extract was evaporated to dryness at 45°C under a nitrogen stream using a Zymark TurboVap LV Evaporator, then reconstituted in 0.0625 volumes methanol to concentrate the extract 16-fold. The structures of the products were confirmed by LC/MS, Method A. A 4.0 min retention time peak was observed as the major component, with m/z value of 720 $(M+H)^+$, 15 required for 13-methyl-erythromycin A ($C_{36}H_{65}NO_{13}$). A second peak was observed with a retention time of 6.4 min and with m/z value of 704 $(M+H)^+$, required for 13-methyl-erythromycin B ($C_{36}H_{65}NO_{12}$). 20 25

30 **Example 21**

Construction of plasmid pJLK114

Plasmid pJLK114 is a pCJR24 based plasmid containing a 35 PKS gene comprising the ery loading module, the first and the second extension modules of the ery PKS and the ery chain-terminating thioesterase except that the DNA segment between the end of the acyltransferase and the beginning of the ACP of the second ery extension module

has been substituted by a synthetic oligonucleotide linker containing the recognition sites of the following restriction enzymes: AvrII, BglII, SnaBI, PstI, SpeI, NsiI, Bsu36I and HpaI. It was constructed via several
5 intermediate plasmids as follows (Figure 6).

Construction of plasmid pJLK02

10 The approximately 1.47 kbp DNA fragment of the eryAI gene of *S. erythraea* was amplified by PCR using as primers the synthetic oligonucleotides:

5'-TACCTAGGCCCGGGCCGACTGGTCGACCTGCCGGGTT-3' and

5'-ATGTTAACCGGTCTGCGCAGGCTCTCCGTCT-3' and plasmid pNTEP2

(Oliynyk, M. *et al.*, Chemistry and Biology (1996) 3:833-
15 839; WO98/01546) as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent
20 *E. coli* DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK02 was identified by its restriction pattern and DNA sequencing.

Construction of plasmid pJLK03

25

The approximately 1.12 kbp DNA fragment of the eryAI gene of *S. erythraea* was amplified by PCR using as primers the synthetic oligonucleotides:

5'-ATGTTAACCGGTCTGCGCGTGCCGAGCGGAC-3' and

5'-CTTCTAGACTATGAATTCCTCCGCCCAGC-3' and plasmid pNTEPH as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK03 was identified by its restriction pattern and DNA sequencing.

Construction of plasmid pJLK04

Plasmid pJLK02 was digested with PstI and HpaI and the 1.47 kbp insert was ligated with plasmid pJLK03 which had been digested with PstI and HpaI. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK04 was identified by its restriction pattern.

Construction of plasmid pJLK05

Plasmid pJLK01 (PCT/GB97/01819) was digested with PstI and AvrII and the 460 bp insert was ligated with plasmid pJLK04 which had been digested with PstI and AvrII. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK05 was identified by its restriction pattern.

Construction of plasmid pJLK07

Plasmid pJLK05 was digested with ScaI and XbaI and plasmid pNTEPH was digested with NdeI and ScaI and these two fragments were ligated with plasmid pCJR24 which had been digested with NdeI and XbaI. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK07 was identified by its restriction pattern.

Construction of plasmid pJLK114

The two synthetic oligonucleotides Plf and Plb (Figure 7) were each dissolved in TE-buffer. 10 μ l of each solution (0.5nmol/ μ l) were mixed and heated for 2 minutes to 65C and then slowly cooled down to room temperature. Plasmid pJLK07 was digested with AvrII and HpaI and ligated with the annealed oligonucleotides. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK114 was identified by its restriction pattern.

Plasmid pJLK117 is a pCJR24 based plasmid containing a PKS gene comprising the ery loading module, the first and the second extension modules of the ery PKS and the ery chain-terminating thioesterase except that the DNA segment between the end of the acyltransferase and the

beginning of the ACP of the second ery extension module has been substituted by a synthetic oligonucleotide linker containing the recognition sites of the following restriction enzymes. AvrII, BglII, SnaBI, PstI, SpeI, NsiI, Bsu36I and NheI.

It was constructed via several intermediate plasmids as follows (Figure 6).

10 Construction of plasmid pJLK115

Plasmid pJLK114 was digested with NdeI and XbaI and the approximately 9.9 kbp insert was ligated with plasmid pUC18 which had been digested with NdeI and XbaI. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK115 was identified by its restriction pattern.

20 Construction of plasmid pJLK116

Plasmid pJLK13 (PCT/GB97/01819) was digested with Bsu36I and XbaI and the 1.1 kbp fragment was ligated with plasmid pJLK115 which had been digested with Bsu36I and XbaI. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK116 was identified by its restriction pattern.

Construction of plasmid pJLK117

Plasmid pJLK116 was digested with NdeI and XbaI and the 9.9 kbp fragment was ligated with plasmid pCJR24 which had been digested with NdeI and XbaI. The ligation mixture was used to transform electrocompetent *E. coli* DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK117 was identified by its restriction pattern.

Example 11

Construction of plasmid pJLK29

Plasmid pJLK29 is a pJLK117 based plasmid except that the DNA fragment encoding the reductive loop of module 10 of the rap PKS has been inserted into the mcs. It was constructed via several intermediate plasmids as follows. (Figure 5)

Construction of plasmid pJLK121.1

The approximately 2.2 kbp DNA segment of the rapB gene of *S. hygroscopicus* encoding the reductive loop of module 10 was amplified by PCR using as primers the synthetic oligonucleotides:

5'-TAAGATCTTCCGACGTACGCGTTCCAGC-3' and

5'-ATGCTAGCCACTGCGCCGACGAATCACCGGTGG-3' and as template an approximately 7 kbp fragment, which has been obtained

by digestion of cosmid cos 26 (Schwecke, T. et al. (1995) Proc. Natl. Acad. Sci. USA 92:7839-7843) with ScaI and SphI. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had
5 been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK121.1 was identified by
10 its restriction pattern and DNA sequencing.

Construction of plasmid pJLK29

Plasmid pJLK121.1 was digested with BglII and NheI and
15 the 2.2 kbp fragment was ligated with plasmid pJLK117 which had been digested with BglII and NheI. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK29 was
20 identified by its restriction pattern.

Example 24

Construction of Plasmid pJLK50

25 The approximately 6.1 kbp DNA segment of the erythromycin PKS gene cluster of *S. erythraea* encoding the DNA fragment from the beginning of the ACP of module 2 to the beginning of the ACP of module 3 was amplified by PCR

using as primers the synthetic oligonucleotides:

5'-TACCTGAGGGACCGGCTAGCGGGTCTGCCGCGTG-3' and

5'-ATGCTAGCCGTTGTGCCGGCTCGCCGGTTCGGTCC-3' and plasmid

pBAM25 (published pBK25 by Best, D J et al. Eur J Biochem

5 (1992) 204: 39-49) as template. The PCR product was

treated with T4 polynucleotide kinase and then ligated

with plasmid pUC18, which had been linearised by

digestion with SmaI and then treated with alkaline

phosphatase. The ligation mixture was used to transform

10 electrocompetent E. coli DH10B cells and individual

colonies were checked for their plasmid content. The

desired plasmid pJLK50 was identified by its restriction

pattern and DNA sequencing.

15 **Example 25**

Construction of *S.erythraea* strain JLK10

Strain JLK10 is a variant of strain NRRL2338 in which the
reductive loop of ery module 2 (i.e. the KR domain) is

20 replaced by the reductive loop of the rapamycin module

10. It was constructed using plasmid pJLK54 which was

constructed as follows.

Construction of plasmid pJLK54

25

Plasmid pJLK54 is a pJLK29 based plasmid containing a PKS
gene comprising the ery loading module, the first, the
second and the third extension modules of the ery cluster
and the ery chain-terminating thioesterase except that

the DNA segment between the end of the acyltransferase and the beginning of the ACP of the second ery extension module has been substituted by the equivalent segment of module 10 of the rapamycin PKS.

5 It was constructed as follows.

Plasmid pJLK50 was digested with NheI and the 6.1 kbp insert was ligated with plasmid pJLK29 which had been digested with NheI. The ligation mixture was used to
10 transform electrocompetent *E. coli* DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK54 was identified by its restriction pattern.

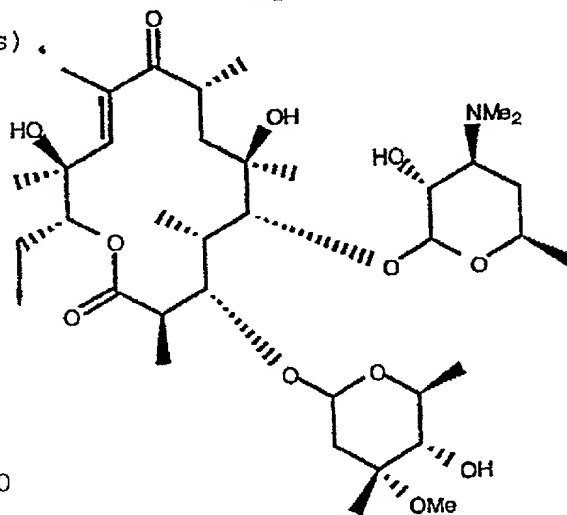
15 Use of plasmid pJLK54 for construction of *S. erythraea* NRRL2338/pJLK54 and the production of TKL derivatives

Approximately 5 μ g plasmid pJLK54 were used to transform protoplasts of *S. erythraea* NRRL2338 and stable
20 thiostrepton resistant colonies were isolated. From several colonies total DNA is obtained and analysed by Southern blot hybridisation, to confirm that the plasmid has integrated into the TE.

25 **Construction of *S.erythraea* strain JLK10 and its use in production of 13-methyl-10,11-dehydro-erythromycin A**

S. erythraea strain JLK10 is a mutant of *S. erythraea* NRRL2338 in which the 'reductive loop' of ery module 2 i.e. the ketoreductase domain is substituted by the

'reductive loop' of rapamycin module 10. It was constructed starting from *S. erythraea* NRRL2338 into which plasmid pJLK54 had been integrated. *S. erythraea* NRRL2338/pJLK54 was subjected to several rounds of non-selective growth which resulted in second crossover concomitant with the loss of the integrated plasmid. Clones in which replacement of the erythromycin gene coding for DEBS1 with the mutant version had occurred, were identified by Southern blot hybridisation. One of these was named *S. erythraea* strain JLK10 and was used to inoculate SM3 medium (eryP medium gave similar results), and allowed to grow for seven to ten days at 28-30°C. After this time the broth was centrifuged and the pH of the supernatant adjusted to pH 9. The supernatant was then extracted three times with an equal volume of ethyl acetate and the solvent was removed by evaporation. Products were analysed by HPLC/MS, MS/MS and ¹H-NMR. The following macrolide C-13 methyl erythromycin A was identified (accompanied by products of incomplete processing by post-PKS enzymes).



Example 26

Construction of plasmid pPFL50

Plasmid pPFL50 is a pPFL43-based plasmid from which a DNA

fragment encoding KR1 (in part), ACP1 and module 2 of the erythromycin PKS and the erythromycin TE, has been removed. It was constructed as follows. Plasmid pPFL43 was digested with SfuI and XbaI to remove a 6.5 kb
5 fragment. The 5' overhangs were filled in with Klenow fragment DNA Polymerase I and the plasmid was recircularised. The ligation mixture was used to transform electrocompetent *E. coli* DH10B cells and individual colonies were checked for their plasmid
10 content. The desired plasmid pPFL50 was identified by its restriction pattern.

Construction of *S. erythraea* JLK10/pPFL50

Approximately 5 µg plasmid pPFL50 were used to transform
15 protoplasts of *S. erythraea* strain JLK10 and stable thiostrepton resistant colonies were isolated. From several colonies total DNA was obtained and analysed by Southern blot hybridisation, to confirm that the plasmid had integrated into the homologous chromosomal DNA
20 region. *S. erythraea* strain JLK10/pPFL50 was used to inoculate SM3 medium containing 5 µg/ml thiostrepton (eryP medium containing 5 µg/ml thiostrepton gave similar results) and allowed to grow for seven to ten days at 28-30°C. After this time the broth was centrifuged and the
25 pH of the supernatant adjusted to pH 9. The supernatant was then extracted three times with an equal volume of ethyl acetate and the solvent was removed by evaporation. Products were analysed by HPLC/MS, MS/MS and 1H-NMR. The macrolide C-13 methyl 10,11-dehydro-erythromycin A was

identified (accompanied by products of incomplete processing by post-PKS enzymes)

Construction of *S. erythraea* NRRL2338/pPFL50

- 5 Approximately 5 µg plasmid pPFL50 were used to transform protoplasts of *S. erythraea* NRRL2338 and stable thiostrepton resistant colonies were isolated. From several colonies total DNA was obtained and analysed by Southern blot hybridisation, to confirm that the plasmid
- 10 had integrated into the homologous region of the chromosomal DNA. *S. erythraea* NRRL2338/pPFL50 was used to inoculate SM3 medium containing 5 µg/ml thiostrepton (eryP medium containing 5 µg/ml thiostrepton gives similar results) and allowed to grow for seven to ten
- 15 days at 28-30°C. After this time the broth was centrifuged and the pH of the supernatant adjusted to pH 9.5. The supernatant was then extracted three times with an equal volume of ethyl acetate and the solvent was removed by evaporation. Products were analysed by
- 20 HPLC/MS, MS/MS and 1H-NMR. The macrolide C-13 methyl erythromycin A was identified (accompanied by products of incomplete processing by post-PKS enzymes).

Construction of plasmid pCB121

- 25 Plasmid pCB121 is a plasmid containing the monensin loading module and KS of monensin module 1 followed by the erythromycin module 1 AT and part of the erythromycin module 1 KR. It was constructed via several intermediate plasmids as follows.

Construction of plasmid pPFL45

The approximately 1.8 kbp DNA segment of the monensin PKS gene cluster of *Streptomyces cinnamonensis* encoding part of the ACP of the loading module and KS of module 1 was
5 amplified by PCR using as primers the synthetic oligonucleotides:

5'-CGTTCCTGAGGTCGCTGGCCCAGGCGTA-3'

5'-CGAAGCTTGACACCGCGGCGGCGCGG-5'

and a cosmid containing the 5' end of the monensin PKS
10 genes from *S. cinnamonensis* or alternatively chromosomal DNA of *S. cinnamonensis* as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with *Sma*I and then treated with alkaline
15 phosphatase. The ligation mixture was used to transform electrocompetent *E. coli* DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pPFL45 was identified by its restriction pattern.

Construction of plasmid pPFL47

Plasmid pPFL45 was digested with *Nde*I and *Bsu*36I and the approximately 2.6 kbp fragment was ligated into plasmid pPFL43 which had been digested with *Nde*I and *Bsu*36I. The ligation mixture was used to transform electrocompetent *E. coli* DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pPFL47 was identified by its restriction pattern.

Construction of plasmid pCB135

Plasmid pCJR24 was digested with *Hind*III, the 5' overhang was filled in with Klenow fragment DNA Polymerase I and
25 religated. The ligation mixture was used to transform electrocompetent *E. coli* DH10B cells and individual colonies were checked for their plasmid content. The

53

desired plasmid pCB135 was identified by its restriction pattern, lacking the recognition site for HindIII.

Construction of plasmid pKSW1

Plasmid pKS1W is a pNTEP2 (GB97/01810)-derived vector containing a DEBS1TE-derived triketide synthase with the unique restriction sites introduced at the limits of KS1.

- 5 Plasmid pKS1W is obtained via several intermediate plasmids as follows.

Construction of plasmids pMO09, pMO10 and pMO13

- 10 For the PCR amplification for plasmid pMO09, the following synthetic oligonucleotides were used as mutagenic primers, one containing a MunI site and the other a PstI site:

5' -GCGCGCCAATTGCGTGACATCTCGAT- 3'

and 5' -CCTGCAGGCCATCGCGACGACCGCGACCGGTTGCGCCG- 3'

15

For the PCR amplification for plasmid pMO10, the following synthetic oligonucleotides were used as mutagenic primers, one containing a HindIII site and the other an EcoRV site:

20 5' -GTCTCAAGCTTCGGCATCAGCGGCACCAA- 3'

and 5' -CGTGCGATATCCCTGCTCGGCGAGCGCA-3'

- 25 For the PCR amplification for plasmid pMO13, the following synthetic oligonucleotides were used as mutagenic primers, one containing a PstI site and the other a HindIII site:

5' -GATGGCCTGCAGGCTGCCCCGGCGGTGTGAGCA- 3'

and 5' -GCCGAAGCTTGAGACCCCCGCCCCGGCGCGGTTCGC- 3'

PCR was carried out on pNTEP2 (GB97/01810) as template using Pwo DNA polymerase and one cycle of: 96°C (1min); annealing at 50°C (3min); and extension at 72°C (1min), and 25 cycles of: 96°C (1min); annealing at 50°C (1min);
5 and extension at 72°C (1min) in the presence of 10% (vol/vol) dimethylsulphoxide. The products were end-repaired and cloned into pUC18 digested with SmaI and the ligation mixture was transformed into E. coli DH 10B. Plasmid DNA was prepared from individual colonies. The
10 desired plasmids for pMO09 (3.8kbp), pMO10 (3.9 kbp) and pMO13 (4.3 kbp) were identified by their restriction pattern and DNA sequencing.

Construction of plasmid pMO11

15 Plasmid pMO13 was digested with HindIII, and the 1.2 kbp insert was cloned into pMO10 which had been digested with HindIII. The ligation mixture was transformed into E. coli DH 10B. The desired plasmid (5.0 kbp) was identified by its restriction pattern and designated
20 pMO11.

Construction of plasmid pMO12

Plasmid pMO09 was digested with PstI, and the 1.6 kbp insert was cloned into pMO11 which had been digested with
25 PstI. The ligation mixture was transformed into E. coli DH 10B. The desired plasmid (6.6 kbp) was identified by its restriction pattern and designated pMO12.

Construction of pKS1W

Plasmid pM012 was digested with MunI and EcoRV, and the 3.9 kbp fragment was cloned into pNTEPH (see below) which had been digested with MunI and EcoRV. The ligation mixture was transformed into E. coli DH 10B. The desired
5 plasmid (13. kbp) was identified by its restriction pattern and designated pKS1W.

Construction of pNTEPH

Plasmid pNTEPH was obtained from pNTEP2 by removing the
10 HindIII site. pNTEP2 was digested with HindIII, the 5' overhang was filled in with Klenow Fragment DNA Polymerase I and religated. The desired plasmid (13.6 kbp) was identified by its restriction pattern.

15 Construction of plasmid pCB136

Plasmid pKSW1 was digested with NdeI and XbaI and the approximately 11.2 kbp fragment was ligated with plasmid pCB135 which had been digested with NdeI and XbaI. The ligation mixture was used to transform electrocompetent
20 E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pCB136 was identified by its restriction pattern.

Construction of plasmid pCB137

25 Plasmid pCB136 was digested with SfuI and XbaI to remove a 6.5 kb fragment, the 5' overhangs were filled in with Klenow Fragment DNA Polymerase I and religated. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked

for their plasmid content. The desired plasmid pCB137 was identified by its restriction pattern.

Construction of plasmid pCB121

5 Plasmid pPFL47 was digested with NdeI and HindIII and the approximately 4.4 kbp insert was ligated with plasmid pCB137 which had been digested with NdeI and HindIII. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked
10 for their plasmid content. The desired plasmid pCB121 was identified by its restriction pattern.

Example

Construction of *S. erythraea* JLK10/pCB121

15 Approximately 5 µg plasmid pCB121 were used to transform protoplasts of *S. erythraea* JLK10 and stable thiostrepton resistant colonies were isolated. From several colonies total DNA was obtained and analysed by Southern blot hybridisation, to confirm that the plasmid had integrated
20 into the homologous chromosomal DNA region. *S. erythraea* strain JLK10/pCB121 was used to inoculate SM3 medium containing 5 µg/ml thiostrepton (eryP medium containing 5 µg/ml thiostrepton gave similar results) and allowed to grow for seven to ten days at 28-30°C. After this time
25 the broth was centrifuged and the pH of the supernatant adjusted to pH 9. The supernatant was then extracted three times with an equal volume of ethyl acetate and the solvent was removed by evaporation. Products were

analysed by HPLC/MS, MS/MS and ¹H-NMR. The macrolide C13-methyl-10,11-dehydro-erythromycin A was identified (accompanied by products of incomplete processing by post-PKS enzymes):

5

Example

Construction of *S. erythraea* NRRL2338/pCB121

Approximately 5 µg plasmid pCB121 were used to transform protoplasts of *S. erythraea* NRRL2338 and stable
10 thiostrepton resistant colonies were isolated. From several colonies total DNA was obtained and analysed by Southern blot hybridisation, to confirm that the plasmid had integrated into the homologous chromosomal DNA region. *S. erythraea* NRRL2338/pPFL50 was used to
15 inoculate SM3 medium containing 5 µg/ml thiostrepton (eryP medium containing 5 µg/ml thiostrepton gave similar results) and allowed to grow for seven to ten days at 28-30°C. After this time the broth was centrifuged and the pH of the supernatant adjusted to pH=9.. The supernatant
20 was then extracted three times with an equal volume of ethyl acetate and the solvent was removed by evaporation. Products were analysed by HPLC/MS, MS/MS and ¹H-NMR. The macrolide C13-erythromycin A was identified (accompanied by products of incomplete processing by post-PKS
25 enzymes):

Although the present invention is illustrated by the examples listed above, they should not be regarded as limiting the scope of the invention. The above

descriptions illustrate for the first time the construction of a Type I PKS gene assembly containing a wholly or partly heterologous KSq-containing loading module and its use to obtain polyketide products of utility as synthetic intermediates or as bioactive materials such as antibiotics. It will readily occur to the person skilled in the art that a wholly or partly heterologous KSq-containing loading module from other PKS gene sets could be used to replace the loading module of DEBS, or indeed into a quite different PKS gene assembly. It will also readily occur to the person skilled in the art that that the additional specificity provided by the more efficient discrimination made between methylmalonyl-CoA and malonyl-CoA by an ATq, followed by specific decarboxylation by a KSq, is preferable to the imperfect discrimination between propionyl-CoA and acetyl-CoA that is a feature of the DEBS loading module and of many other PKS loading modules, in that it maximises the production of a single product rather than a mixture differing from each other in the nature of the starter unit. The avoidance of such mixtures increases yields and avoids the need for tedious and difficult separation procedures.

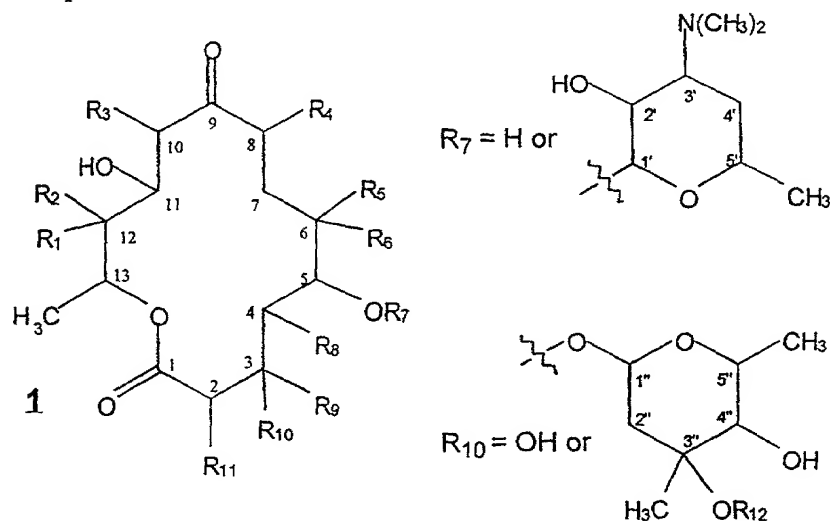
Claims

1. A 14-member macrolide which incorporates an acetate starter unit so that it has a 13-methyl substituent, with the proviso that it is not
 5 norerythromycin C, 6-deoxy-15-norerythromycin B or 6-deoxy-15-norerythromycin D.

2. 15-norerythromycin A.

10 3. 15-norerythromycin B.

4. A compound of the formula 1:



15

or a pharmaceutically acceptable salt thereof, wherein:

R_1 is H or OH; R_2 - R_4 are each independently H, CH_3 , or CH_2CH_3 ; R_5 is H or OH; and R_6 is H, CH_3 , or CH_2CH_3 ; R_7 is H or desosamine; R_8 is H, CH_3 , or CH_2CH_3 ; R_9 is OH, mycarose (R_{12} is H), or cladinose (R_{12} is CH_3), R_{10} is H; or $R_9 = R_{10}$

20

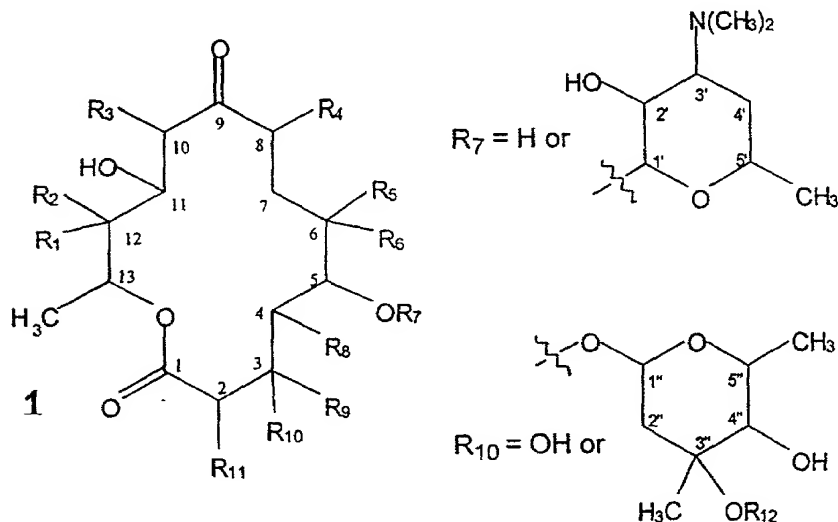
60

= O; and R_{11} is H, CH_3 , or CH_2CH_3 , with the proviso that when R_2 - R_4 are CH_3 , R_6 is CH_3 , R_8 is CH_3 , and R_{11} is CH_3 , then R_1 and R_5 are not H and R_{12} is not H; or also when R_2 - R_4 are CH_3 , R_6 is CH_3 , R_8 is CH_3 , and R_{11} is CH_3 , then R_1 and R_5 are not OH and R_{12} is not H.

5. A compound according to claim 4 wherein R_1 is OH; R_2 - R_4 are CH_3 ; R_5 is OH; R_6 is CH_3 , R_7 is desosamine; R_8 is CH_3 ; R_9 is cladinose (R_{12} is CH_3); and R_{11} is CH_3

6. A compound according to claim 4 wherein R_1 is H; R_2 - R_4 are CH_3 ; R_5 is OH; R_6 is CH_3 , R_7 is desosamine; R_8 is CH_3 ; R_9 is cladinose (R_{12} is CH_3); and R_{11} is CH_3 .

7. A process for making compounds of the formula 1:



wherein:

R_1 is H or OH; R_2 - R_4 are each independently H, CH_3 , or CH_2CH_3 ; R_5 is H or OH; and R_6 is H, CH_3 , or CH_2CH_3 ; R_7 is H or desosamine; R_8 is H, CH_3 , or CH_2CH_3 ; R_9 is OH, mycarose

(R₁₂ is H), or cladinose (R₁₂ is CH₃), R₁₀ is H; or R₉ = R₁₀ = O; and R₁₁ is H, CH₃, or CH₂CH₃

8. A process for making compound of the formula 1 as set out in claim 7 wherein R₁ is OH; R₂-R₄ are CH₃; R₅ is OH; R₆ is CH₃, R₇ is desosamine; R₈ is CH₃; R₉ is cladinose (R₁₂ is CH₃); and R₁₁ is CH₃

9. A process for making compound of the formula 1 as set out in claim 7 wherein R₁ is H; R₂-R₄ are CH₃; R₅ is OH; R₆ is CH₃, R₇ is desosamine; R₈ is CH₃; R₉ is cladinose (R₁₂ is CH₃); and R₁₁ is CH₃

10. A system for producing a 14-membered macrolide incorporating an acetate starter unit, said system comprising DNA encoding and arranged to express a PKS multienzyme which comprises a loading module and a plurality of extension modules; wherein in the expressed multienzyme, said loading module is adapted to load a malonyl residue and then to effect a decarboxylation of the loaded residue to provide an acetate starter unit which is transferred to an adjacent one of said extension modules; and wherein the extension modules, or at least one thereof, are not naturally associated with a loading module that effects decarboxylation.

11. A system according to claim 10 wherein the macrolide is a compound of formula 1 as defined in any of claims 4-9.

12. A system according to claim 10 or 11 wherein said adjacent extension module to which the acetate starter is transferred is not naturally associated with a loading module that effects decarboxylation.

13. A system according to claim 10, 11 or 12 wherein the decarboxylating functionality of the loading module is provided by a ketosynthase-type domain having a glutamine residue in the active site.

14. A system according to claim 10, 11 or 12 wherein the decarboxylating functionality of the loading module is provided by a CLF-type domain.

15. A system according to claim 14 wherein the CLF-type domain is substantially as any shown in Fig 2.

16. A system according to any of claims 10-15 wherein the loading module's loading functionality is provided by an acyltransferase-type domain having an arginine residue in the active site.

17. A system according to any of claims 10-16 wherein the loading module includes an acyl carrier protein.

18. A system according to any of claims 10-13, 16 or 17 wherein at least the KS₀ domain of said loading module corresponds to the loading module of the PKS multienzyme of oleandomycin, spiramycin, niddamycin, methymycin, or monensin.

19. A PKS multienzyme as expressible by the DNA of the system of any of claims 10-18 or a variant having the ability to synthesise a compound of formula 1.

20. Nucleic acid encoding the PKS multienzyme of claim 19.

21. A vector containing nucleic acid as defined in claim 20.

22. A transformant organism comprising a system according to any of claims 10-18.

23. A process according to claim 7, 8, or 9 which comprises culturing an organism according to claim 22 and recovering a compound of formula 1.

24. A process according to claim 23 wherein said macrolide is a compound of formula 1 as defined in any of claims 4-9.

25. A system, organism or process according to any of
claims 10-24 wherein the plurality of extension modules
corresponds to the extension modules of a PKS selected
s from erythromycin, narbomycin, pikromycin, lankamycin,
kujimycin or megalomycin or a mutant or variant thereof
able to direct synthesis of a macrolide.

The erythromycin PKS

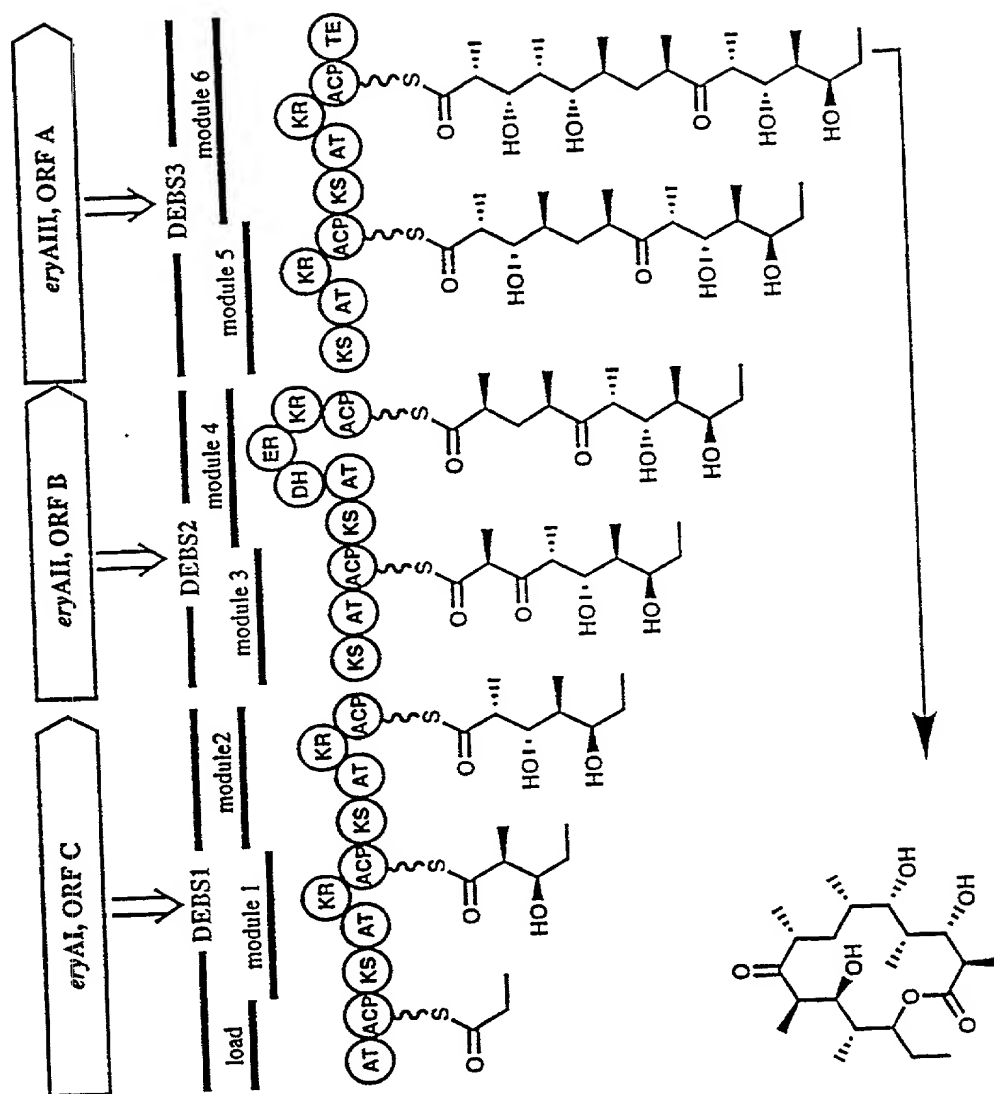


Fig. 1

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09/720841-091304

KCLFDAU	-----MVTGLGIVAPNGLGVGAIWDAVLNNGRNGIGPLR
KCLFPEU	MTGTAARTASSQLHASAPAGRRGLRGRAVVTGLGIVAPNGLGVGAYWDAVLNNGRNGIGPLR
KCLFACT	-----MSVLTITGVGVVAPNGLGLAPYWSAVLDGRHGLGPVT
KCLFHIR	-----MSTWVTGMGVVAPNGLGADHWAATLKGRHGISRLS
KCLFGRA	-----MSTPDRRRRAVVTGLSVAAPGGLGTERYWKSLLTGENGLAELS
KCLFNOG	-----MTAAVVVTGLGVVAPTGLGVREHWSSTVRGASAIGPVT
KCLFTCM	-----MSAPAPVVVTGLGIVAPNGLGTEEYWAATLAGKSGIDVIQ
KCLFCIN	-----MTP-VAVTGMGLAAPNGLGRPTTGRPPWAPRAASAAS
KCLFVNZ	-----MSASVVVTGLGVAAPNGLGREDFWASTLGGKSGIGPLT
KCLFWHIE	-----MSGPQRTGTGGSSRAVVTGLGVLSPHGTGVEAHKAVADGTSSLG PVT
KSGRA	-----MTRRVVITGVGVRAVPGGSGTKFDFWDLTSGRTATRIS
KSHIR	-----MTRRVVITGVGVRAVPGGSGTKFDFWDLTSGRTATRIS
KSACT	-----MKRRVVTITGVGVRAVPGGSGTKFDFWDLTSGRTATRIS
KSCIN	-----MTQRRVAITGIEVLAPGGLGRKEFWQLLSEGRATATRGIT
KSVNZ	-----MTARRVVTITGIEVLAPGGLGRKEFWQLLSEGRATATRGIT
KSNOG	-----MKEVINRRVVTITGIGIVAPDATGVKPFWDLLTAGRTATRTIT
KSTCM	-----MTRHAERVVITGIGIVAPDATGVKPFWDLLTAGRTATRTIS
KSDAU	-----MNRRVVTITGMGVVAPGAIGIKSFWEALLSGTTATRAIT
KSPEU	-----MNRRIVITGIGIVAPGAIGIKSFWEALLSGTTATRAIS
KSWHI	-----MTRRRVAVTIGIVVAPGGIGTPQFWLLSEGRATATRIS

: ** : : * . *

KCLFDAU	RFADDGRLGRLAGEVSDFVP-EDHLPKRLLVQTDPMQMTALAAAEWALREAGCAPSS--
KCLFPEU	RFTGDGRLGRLAGEVSDFVP-EDHLPKRLLAQTDPMQY-ALAAAEWALRESGCSFSS--
KCLFACT	RFDVSRYPATLAGQIDDFHA-PDHI PGRLLPQTDPSTRL-ALTAADWALQADAPES-L
KCLFHIR	RFDPTGYPAELAGQVLDFA-TEHLPKRLLPQTDVSTRF-ALAAAALADAEVDPAE-L
KCLFGRA	RFDASRYPSRLAGQIDDFEA-SEHLPKRLLPQTDVSTRY-ALAAADWALADAGVGPESGL
KCLFNOG	RFDAGRYPSKLAGEVPGFVP-EDHLPKRLLPQTDHMTL-ALVAADWAFQDAAVDPK-L
KCLFTCM	RFDPHGYPVVVGGEVLAFDA-AAHLPGRLLPQTDRTQH-ALVAAEWALADAGLEPEK-Q
KCLFCIN	RFDPSGYPAQLAGEIPGFRA-AEHLPGRLLPQTDRTVTRL-SLAAADWALADAGVEVAA-F
KCLFVNZ	RFDPTGYPARLAGEVPGFAA-EEHLPKRLLPQTDRTMTRL-ALVAADWALADAGVRPEE-Q
KCLFWHIE	REGCAHLPLRVAGEVHGFDA-AETVEDRFLVQTDRTFHF-ALSATQHADARFGRADV
KSGRA	FFDASPFRSRIAGEI-DFDAVAEGFSPREVRMDRATQF-AVACTRDALADSGLDIGA-L
KSHIR	FFDPTPNRSQIAAEC-DFDPEHEGLSPREIRMDRAAQF-AVVCTRDAVADSGLEFEQ-V
KSACT	FFDPSPYRSQVAAEA-DFDPVAEGFGPRELDRMDRASQF-AVACAREAFASGLDPDT-L
KSCIN	FFDPAPFRSKVAAEA-DFCGLNGLSPQEVRRMDRAAQF-AVVATAR-AVEDSGAELAA-H
KSVNZ	FFDPTPFRSRVAAEI-DFDPEAHGLSPQEIIRMDRAAQF-AVVAAR-AVADSGIDLAA-H
KSNOG	AFDPSPFRRSRIAAEC-DFDPLAEGLTQQIIRMDRATQF-AVVSARESLDSGLDLGA-L
KSTCM	LFDAAPYRSRIAGEI-DFDPIGEGLSPRQASTYDRATQL-AVVCAREALKDSGLDPAA-V
KSDAU	TFDATPFRSRIAAEC-DFDPVAAGLSAEQARRLDRAQF-ALVAGQEALTDGLRIGE-D
KSPEU	TFDATPFRSRIAAEC-DFDPVAAGLSAEQARRLDRAQF-ALVAGQEALADSGLRIDE-D
KSWHI	LFDP SGLRSQIAAEC-DFEPSDHGLGLATAQRCDRYVQF-ALVAAEAVRDANLDMNR-E

: . . : * . * : : : . :

Fig 2A

09/720841

KCLFDAU
KCLFPEU
KCLFACT
KCLFHIR
KCLFGRA
KCLFNOG
KCLFTCM
KCLFCIN
KCLFVNZ
KCLFWHIE
KSGRA
KSHIR
KSACT
KSCIN
KSVNZ
KSNOG
KSTCM
KSDAU
KSPEU
KSWHI

-PLEAGVITASASGGFASGQRELQNLWSKG-----PAHVSAYMSFAWFY-AVNTGQIAIR
-PLEAGVITASASGGFAFGQRELQNLWSKG-----PAHVSAYMSFAWFY-AVNTGQIAIR
TDYDMGVVTANACGGFDFTHREFRKLWSEG-----PKSVSVYESFAWFY-AVNTGQISIR
PEYGTGVITSNATGGFEFTHREFRKLWAQG-----PEFVSUYESFAWFY-AVNTGQISIR
DDYDLGVVTSTAQGGFDFTHREFHKLWSQG-----PAYVSVYESFAWFY-AVNTGQISIR
PEYGVGVVTASSAGGFEGFHRELQNLWSLG-----PQYVSAYQSFAYFY-AVNTGQVSIR
DEYGLGVLTAAAGAGGFEGQREMOKLWGTG-----PERVSAYQSFAYFY-AVNTGQISIR
DPLDMGVVTASAGGFEGQDELQKLLGQG-----QPVL SAYQSFAYFY-AVNSGQISIR
DDFDMGVVTASASGGFEGQDELQKLLWSQG-----SQYVSAYQSFAYFY-AVNSGQISIR
SPYSVGVTAAAGSGGGEFGQRELQNLWGHG-----SRHVGPYQSIAYFY-AASTGQVSIR
DPSRIGVALGSASASATSLENEYLVMSDSGREWLVDPAHLSPMFMDYLSPGVMPAEVAWA
PPERIGVSLGSASAAAATSLQEYLVLSDDGGREWQVDPAYLSAHMFDYLSPGVMPAEVAWT
PPEYGVVSLGSASAAAATSLEREYLLLSDDSGRDWEVDAAWLSRHMFDYLVPSVMPAEVAWA
PPHRI GVVVGSASGATMGLDNEYRVVSDGGRLDLVDHRYAVPHLYNYLVPSFAAEVAWA
DPYRVGVTVGSASGATMGLDEEYRVVSDGGRLDLVDHAYAVPHLYDYMPVPSFAAEVAWA
DASRTGVVVGSAVGCTTSLEEEYAVVSDSGRNWLVDDGYAVPHLEFDYFVPSSIAAEVAHD
NPERIGVSI GTAVGCTTGLDREYARVSEGGSRWLVDHTLAVEQLFDYFVPTSICREVAWE
SAHRVGVCVGTAVGCTQKLESEYVALSAGGANWVDPHRAPELYDYFVPSSIAAEVAWL
SAHRVGVCVGTAVGCTQKLESEYVALSAGGAHWVDPGRGSPELYDYFVPSSIAAEVAWL
DPWRAGATLGTAVGGTTRLEHDYVLVSEGRSRWDVDRRSEPHLERAFPTATLSSAAVEE

* : : *

KCLFDAU
KCLFPEU
KCLFACT
KCLFHIR
KCLFGRA
KCLFNOG
KCLFTCM
KCLFCIN
KCLFVNZ
KCLFWHIE
KSGRA
KSHIR
KSACT
KSCIN
KSVNZ
KSNOG
KSTCM
KSDAU
KSPEU
KSWHI

-HDLRGPVGVVVAEQAGGLDALAHAR-RKVRGGAE-LIVSGAMDSSLCP-YGMAAQVRS
-HDLRGPVGVVVAEQAGGLDALAHAR-RKVRGGAE-LIVSGAVDSSLCP-YGMAAQVRS
-HGMRGPSALVAEQAGGLDALGHAR-RTIRRGTP-LVVSAGVDSALDP-WGWVSQIASG
-HGLRGPVSVLVAEQAGGLDAVGHGG--AVRNGTP-MVVTGGVDSSFDLP-WGWVSHVSSG
-NTMRGPSAALVGEQAGGLDAIGHAR-RTVRRGPG-WCSAVASTRRSTR-GASSSQLSGG
-HGLRGPVGVLVTEQAGGLDALQAR-RQLRRGLP-MVAVAGVDGSPCP-WGWVAQLSSG
-HGMRGHSSVVFTEQAGGLDAAAHAA-RLLRKGTLTALTGGCEASLCP-WGLVAQIPSG
-HGMKGPSGVVSDQAGGLDALAQAR-RLVRKGTP-LIVCGAVEPRSAAGAGSPSSPAGG
-NGMKGPSGVVSDQAGGLDAVAQAR-RQIRKGT-TR-LIVSGGVDSALCP-WGWVAHVASD
-NDFKGPCGVVADEAGGLDALAHAA-LAVRNGTD-TVVCGATEAPLAP-YSTVCQLGYP
-AGAEGPVTMVSDGCTSGLDVSGYAV-QGTREGSADVVAGAADTPVSPITVACFDAIKA
-VGAEGPVAMVSDGCTSGLDVSLSHAC-SLIAEGTTDMVAGAADTPITPITVACFDAIKA
-VGAEGPVTMVSTGCTSGLDVSGNAV-RAIEEGSADVMFAGAADTPITPITVACFDAIRA
-VGAEGPSTVSTGCTSGIDAVGIAV-ELVREGSVDVMVAGAVDAPISPIP-CVLDAIKA
-VGAEGPNTVSTGCTSGLDVSGYARGELIREGSADVMVLAGSSDAPISPIITMACFDAIKA
RIGAEGPVSLVSTGCTSGLDVAGRAA-DLIAEGAADVMVLAGATEAPISPIITVACFDAIKA
-AGAEGPVTVSTGCTSGLDVAGVGT-ELIRDGRADVVCATDAPISPIITVACFDAIKA
-AGAEGPVNIVSAGCTSGIDVSGYAC-ELIREGTVDMVLAGGVDAPITPITVACFDAIRV
-AGAEGPVNIVSAGCTSGIDVSGYAC-ELIREGTVDMVAGGVDAPITPITVACFDAIRA
-FGVRGPVQTVSTGCTSGLDVAGYAY-HAVAEGRVDCVLAGAADSPISPIITMACFDAIKA

* *

KCLFDAU
KCLFPEU
KCLFACT
KCLFHIR
KCLFGRA
KCLFNOG
KCLFTCM
KCLFCIN
KCLFVNZ
KCLFWHIE

RLSGSDPTAGYLPFDRAAGHVPGEG-GAILAVEDAERVAERG-GKVYGSIAGT-ASFD
RLSGSDNPTAGYLPFDRAAGHVPGEG-GAILTVEDAERAAERG-AKVYGSIAGYGASFD
RJSTATDPDRAYLPFDRAAGHVPGEG-GAILVLEDSAAAEARGRHADYAGELAGCASTFD
RVSRATDPGRAYLPFDVAANGYVPGEG-GAILLLEDAESAKARG-ATGYGEIAGYAAATFD
LVSTVADPERAYLPFDVDASGYVPGEG-GAVLIVEDADSARARG---AERIYVRSPLRRD
GLSTSDPPRAYLPFDAAAGHVPGEG-GALLVLESDESARARGVTRWYGRIDGYAATFD
FLSEATDPHDAYLPFDARAAGYVPGEG-GAMLVAERADSARERDAATVYGRIGHASTFD
-MSDSEPNRAYLPFDORDGRGYVPGGGRGVVPPLERAEEAPARG-AEYGE-AGPLARL-
RLSTSEEPARGYLPFDREAQGHVPGEG-GAILVMEAAEAARERG-ARIYGEIAGYGSTFD
ELSRATEPDRAYRPFTEACGFAPAEG-GAVLVVEEEAARERG-ADVRATVAGHAATFT

Fig 2B

09/720841

KSGRA TTPRNDPPAHASRPFDGTRNGFVLAEG-AAMFVLEEEYAAQRRG-AHIYAEVGGYATRSQ
 KSHIR TTPRNDPPAHASRPFDNSRNGFVLAEG-AALFVLEEEHARARG-AHUYAEISGCATRLN
 KSACT TTARNDDPEHASRPFDGTRDGFVLAEG-AAMFVLEEDYDSALARG-ARIHAEISGYATRCN
 KSCIN TTPRHDPATASRPFDSTRNGFVLGEG-AAFFVLEELHSARRRG-AHIYAEIAGYATRSN
 KSVNZ TTNRYDDPAHASRPFDGTRNGFVLGEG-AAVVFVLEELHSARARG-AHIYAEIAGYATRSN
 KSNOG TTPRNDTPAEASRPFDRTNGFVLGEG-AAVVFVLEEFHARRRG-ALVYAEIAGFATRCN
 KSTCM TSANNDPPAHASRPFDNRDGFVLGEG-SAVFVLEELSAARRRG-AHAYAEVRGFATRSN
 KSDAU TSDHNDTPETLA-PFSRSRNGFVLGEG-GAIVVLEEEAAVVRG-ARIYAEIGGYASRGN
 KSPEU TSDHNDTPETASRPFSRSRNGFVLGEG-GAIVVLEEEAAVVRG-ARIYAEIGGYASRGN
 KSWHI TSPNNDPPAHASRPFDADRNGFVMGEG-AAVLVLEEDLEHARARG-ADVCEVSGYATFGN

* ** * . . * . . *

KCLFDAU -PPPGSGRP---SALARAVETALADAGLDRSDIAVVFADGAA-VGELDVAEEALASVFG
 KCLFPEU -PPPGSGRP---SALARAVETALADAGLDGSDIAVVFADGAA-VPELDAAEEALASVFG
 KCLFACT -PAPGSGRP---AGLERAIRLALNDAGTGPEVDVVFADGAG-VPELDAAEARAIGRVFG
 KCLFHIR -PAPGSGRP---PALRRAI ELALADAELRPEQVDVVFADAG-VAELEDAEAAARELFG
 KCLFGRA -PAPGSGRP---PALGRAELALAEAGLTPADISVVFADGAG-VPELDRAEADTLARLFG
 KCLFNOG -PPPGSGRP---PNLLRAAQAALDDAEVGPVAVDVVFADAG-TPDEDAEADAVRRLFG
 KCLFTCM -ARPGTGRP---TGPARAIRLALAEARVAPEDVDVVFADAG-VPELDRAEAEALAEVFG
 KCLFCIN -PAPHSGRG---STRAHAIRLALDDAGTAPGDIRRVFADGGGGRYPN-DRAEAEALAEVFG
 KCLFVNZ -PRPGSGRE---PGLRKAI ELALADAGAAPGDIDVVFADAAA-VPELDREAEALNAVFG
 KCLFWHIE GAGRWAESR---EGLARAIQALAEAGCRPEEVDVVFADALG-VPEADRAEALALADALG
 KSGRA -AYHMTGLKKGREMAESIRALDEARLDRTAVDYVNAHSGG-TKQNDRHETAAFKRSLG
 KSHIR -AYHMTGLKTDGREMAEIRVALDLARIDPTDIDYINAHSGG-TKQNDRHETAAFKRSLG
 KSACT -AYHMTGLKADGREMAETIRVALDESRTDATDIDYINAHSGG-TQNDRHETAAFKRSLG
 KSCIN -AYHMTGLR-DGAEMAEAIRLALDEARLNPEQVDYINAHSGG-TKQNDRHETAAFKKALG
 KSVNZ -AYHMTGLRDPDGAEMAEIRVALDEARMNPTEIDYINAHSGG-TKQNDRHETAAFKKSLG
 KSNOG -AFHMTGLRDPDGREMAEIGVALAQAGKAPADVYVNAHSGG-TQNDRHETAAFKRSLG
 KSTCM -AFHMTGLRDPDGREMAEITAALDQARRTGDDLHYINAHSGG-TQNDRHETAAFKRSLG
 KSDAU -AYHMTGLRADGAEMAAAI TAALDEARRDP SDVDYVNAHGTA-TQNDRHETSAFKRSLG
 KSPEU -AYHMTGLRADGAEMAAAI TAALDEARRDP SDVDYVNAHGTA-TQNDRHETSAFKRSLG
 KSWHI -AYHMTGLTKEGLEMARAI DTALDMAELDGSADIDYVNAHSGG-TQNDRHETAAVKRSLG

. : : ** : : : * . . * * : : *

Fig 2c

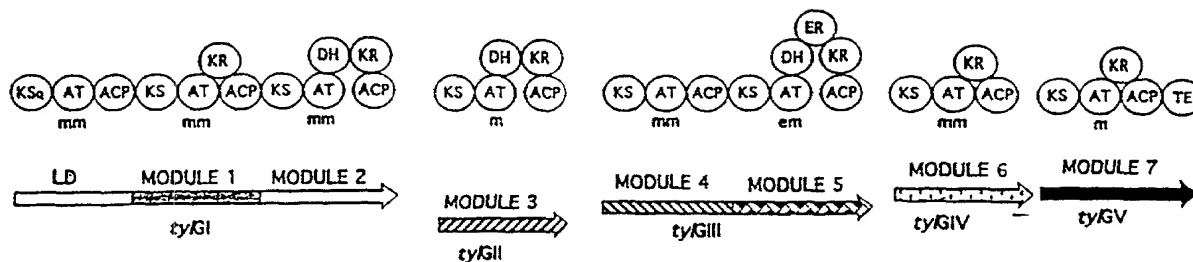
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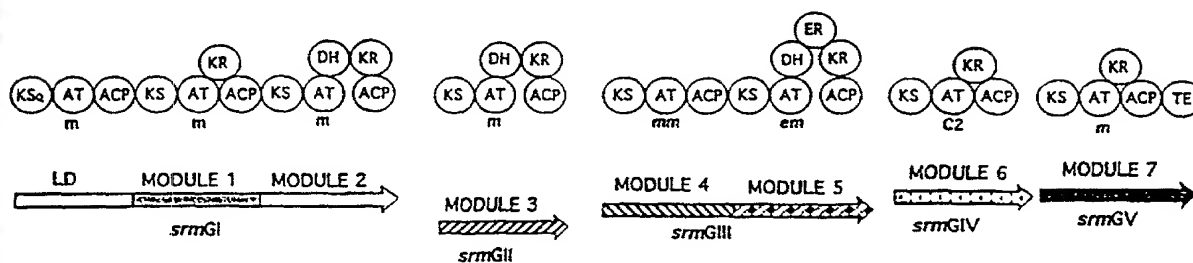
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SUBSTITUTE SHEET (RULE 26)

ORGANISATION OF THE TYLOSIN-PRODUCING POLYKETIDE SYNTHASE



ORGANISATION OF THE SPIRAMYCIN-PRODUCING POLYKETIDE SYNTHASE



ORGANISATION OF THE NIDDAMYCIN-PRODUCING POLYKETIDE SYNTHASE

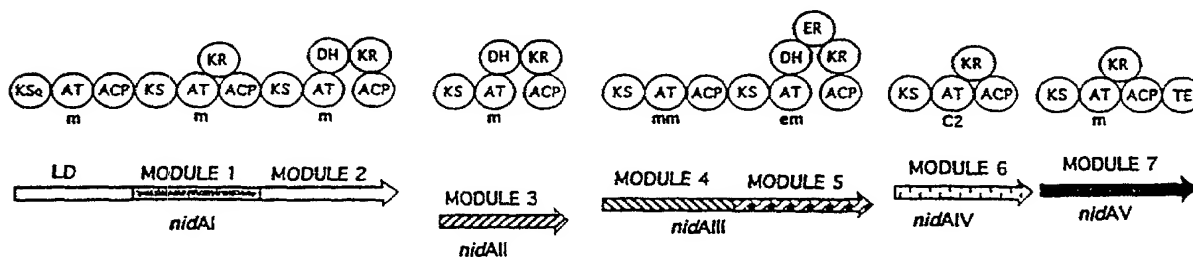


Fig 3

m: malonyl transferase
mm: methylmalonyl transferase
em: ethylmalonyl transferase
C2: unknown C2 unit transferase

Fig. 4A

	1				50
niddamycin	-----	-----	MAGHGDATAQ	KAQDAEKSED	GSDAIAVIGM
platenolide	-----	-----	-----MS	GELAISRSDD	RSDAVAVVGM
monensin	-----	-----	-----MAAS	ASASPSGPSA	GPDPPIAVVGM
oleandomycin	-----	-----	-----	---MHVPGEE	NGHSIAIVGI
tylosin	MSSALRRAVQ	SNCGYGDLMT	SNTAAQNTGD	QEDVDGPDST	HGGEIAVVGM
	51				100
niddam...	SCRFPGAPGT	AEFWQLLSSG	ADAVVTAADG	RRR.....GTIDA
platenol.	ACRFPGAPGI	AEFWKLLTDG	RDAIGRDADG	RRR.....GMIEA
monensin	ACRLPGAPDP	DAFWRLLESG	RSASVSTAPPE	RRRADSGLHG	P...GGYLDR
oleandom	ACRLPGSATP	QEFWRLLESG	ADALDEPPAG	RFPTGSLSSP	PAPRGGLFDS
tylosin	SCRLPGAAGV	EEFWELLRSG	RGMPTREQDDG	TWRAA.....LED
	101				150
niddam...	PADFDAFFFG	MSPREAAATD	PQORLVLELG	WEALEDAGIV	PESLRGEAAS
platenol.	PGDFDAFFFG	MSPREAAETD	PQORLMLELG	WEALEDAGIV	PGSLRGEAVG
monensin	IDGFDADFFH	ISPRAEAVMD	PQORLLELS	WEALEDAGIR	PPTLARSRTG
oleandom	IDTFDADFFN	ISPRAEAGVLD	PQORLALELG	WEALEDAGIV	PRHLRGTRTS
tylosin	HAGFDAGFFG	MNARQAAATD	PQHRLMLELG	WEALEDAGIV	PGDLTGTDTC
	151				200
niddam...	VFVGAMNDY	ATLLH.RAGA	PTDITYTATGL	QHSMIANRLS	YFLGLRGPSL
platenol.	VFVGAMHDDY	ATLLH.RAGA	PVGPHATATGL	QRAMLNRLS	YVLGTRGPSL
monensin	VFVGAFWDDY	TDVLNLRAPG	AVTRHTMTGV	HRSILANRLS	YAYHLAGPSL
oleandom	VFMGAMWDDY	AHLAHARGEA	ALTRHSLTGT	HRGMIANRLS	YALGLOGPSL
tylosin	VFAGVASDDY	A.VLTRRSV	SAGGYTATGL	HRALANRLS	HFLGLRGPSL
	201				250
niddam...	VVDGTQSSSL	VAVALAVESL	RGGTSGIALA	GGVNLVLAEE	GS.AAMERVG
platenol.	AVDTAQSSSL	VAVALAVESL	RAGTSRVAVA	GGVNLVLADE	GT.AAMERLG
monensin	TVDTAQSSSL	VAVHLACESI	RGSDSDIAFA	GGVNLICSPR	TTELAAARFG
oleandom	TVDTGQSSSL	AAVHMACESL	ARGESDLALV	GGVNLVLDPA	GT.TGVERFG
tylosin	VVDSAQSASL	VAVQLACESL	RRGETSLAVA	GGVNLILTEE	ST.TVMERMG
	251				300
niddam...	ALSPDGRCHT	FDARANGYVR	GEGGAIVVLK	PLADALADGD	RVYCVVRGVA
platenol.	ALSPDGRCHT	FDARANGYVR	GEGGAIVVLK	PLADALADGD	PVYCVVRGVA
monensin	GLSAAGRCHT	FDARADGFVR	GEGGGLVVLK	PLAAARRDGD	TVYCVIRGSA
oleandom	ALSPDGRCHT	FDSRANGYVR	GEGGVVVVLK	PTHRALADGD	TVYCEILGSA
tylosin	ALSPDGRCHT	FDARANGYVR	GEGGAVVLK	PLDAALADGD	RVYCVIKGGA
	301				350
niddam...	TGNDGGGPGL	TVPDRAGQEA	VLRAACDQAG	VRPADVRFVE	LHGTGTPAGD
platenol.	VGNDGGGPGL	TAPDREGQEA	VLRAACAQAR	VDPAEVRFVE	LHGTGTPVGD
monensin	VNSDGTDDGI	TLP SGQAQD	VVRLACRRAR	ITPDQVQYVE	LHGTGTPVGD
oleandom	LNNDGATEGL	TVPSARAQAD	VLRQAWERAR	VAPTDVQYVE	LHGTGTPAGD
tylosin	VNNDGGGASL	TPPDREAQEA	VLRQAYRRAG	VSTGAVRYVE	LHGTGTRAGD

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	351		400
niddam...	PVEAEALGAV YGTGRP..AN EPLLVGSVKT NIGHLEGAAG	IAGFVKAAALC	
platenol.	PVEAHALGAV HGSGRP..AD DPLLVGSVKT NIGHLEGAAG	IAGLVKAAALC	
monensin	PIEAAALGAA LGQDAA..RA VPLAVGSAKT NVGHLEAAAAG	IVGLLKTTALS	
oleandom	PVEAEGLGTA LGTARP..AE APLLVGSVKT NIGHLEGAAG	IAGLLKTVLS	
tylosin	PVEAAALGAV LGAGADSGRS TPLAVGSVKT NVGHLEGAAG	IVGLIKATLC	
	401		450
niddam...	LHERALPASL NFETPNPAIP LERLRLKVQT AHAALQPGTG	GGPLLAVGSA	
platenol.	LRERTLPGSL NFATPSPAIP LDQLRLKVQT AAAELPLAPG	GAPLLAGVSS	
monensin	IHHRR LAPSL NFTTPNPAIP LADLGLTVQQ DLADWP..RP	EQPLIAGVSS	
oleandom	IKNRHLPASL NFTSPNPRID LDALRLRVHT AYGWP..SP	DRPLVAGVSS	
tylosin	VRKGELVPSL NFSTPNPDIP LDDLRLRVQT ERQEW.NEED	DRPRVAGVSS	
	451		500
niddam...	FGMGGTNCHV VLEETPGG..	...RQPAE.T	
platenol.	FGIGGTNCHV VLEHLPSR..	...PTPAV.S	
monensin	FGMGGTNGHV VVA....AAP DSVAVPEPVG VPERVEVPEP	VVVSEPVVVP	
oleandom	FGMGGTNCHV VLSELRNAGG DGAGKGPYTG TEDRLGATEA	EKRDPDPATGN	
tylosin	FGMGGTNVHL VIAEAPAAAAG SSGAGGSGAG SGAGISAVSG	VV.....	
	501		550
niddam...	GQADACLFSA SPMLLLSARS EQALRAQAAR LREHL..EDS	GADPLDIAYS	
platenol.	VAAS...LPD VPPLLLSARS EGALRAQAVR LGETV..ERV	GADPRDVAYS	
monensin	TPWP..... VSAHS ASALRAQAGR LRTHLAHRP	TPDAARVGH	
oleandom	GPDPAQDTHR YPALILSARS DAALRAQAER LRHHL.EHSP	GQRLRDTAYS	
tylosin PVVVSGRS RVVVREAAGR LAE..VVEAG	GVGLADVAVT	
	551		600
niddam...	LATTRTRFEH RAAVPCGDPD RLSSALAALA AGOTPRGVRI	GS..TDADGR	
platenol.	LASTRTLFEH RAVVPCGGRG ELVAALGGFA AGRVSGGVRS	GR..A.VPGG	
monensin	LATTRAPLAH RAVLLGGDTA ELLGSLDALA EGAETASIVR	GEAYT..EGR	
oleandom	LATRRQVFER HAVVTGHDRE DLLNGLRDLE NGLPAPQVLL	GRTPTPEPGG	
tylosin	MAD.RSRFGY RAVVLARGEA ELAQLRLALA GGDPDAGVVT	G...AVLDGG	
	601		650
niddam...	LALLFTGQGA QHPGMGQELY TTDPHFAAAL DEVCEELQRC	GTQNLREVFM	
platenol.	VGVLFTHGGA QWVGMRGLY AGGGVFAEVL DEVLSMVGEV	DGRSLRDVFM	
monensin	TAFLFSGQGA QRLGMGRELY AVFPVFADAL DEAFALDVH	LDRPLREIVL	
oleandom	LAFLFSGQGS QPPGMGKRLH QVFPGFRDAL DEVCAELDTH	LGRLL.....	
tylosin	VVGGAAPGGA GAAGGAGAAG GAGGGGVVLV FPGQGTQWVG	MGAGLLGSSE	
	651		700
niddam...	TPDQPD....	LLDRTEYTQP ALFALQTALY	
platenol.	GDVDVDAGAG ADAGAGAGAG VGSGSGSVGG LLGRTEFAQP	ALFALEVALF	
monensin	GETDSGGNVS GENVIGEGA.DHQA LLDQTAYTQP	ALFAIETSLY	
oleandom	.GPEAGPPLR DVMFAERGT.AHSA LLSETHYTQA	ALFALETALF	
tylosin	VFAASMRECA RALSVHVGWD LLEVSGGAG .LERVDVVQP	VTWAVMVSLA	
	701		750
niddam...	RTLTARGETQA HVLVGHVGE ITAAHIAGVL DLPDAARLIT	ARAHVMGQLP	
platenol.	RALEARGVEV SVVLGHVGE VAAATVAGVL SLGDAVRLVV	ARGGLMGGLP	
monensin	RLAASFGLKP DYVLGHVGE IAAAHVAGVL SLPDASALVA	TRGRMLQAVR	
oleandom	RLLVQWGLKP DHLAGHVGE IAAAHAGIL DLSDAALVA	TRGALMRSLP	
tylosin	RYWQAMGVDV AAVVGHSQGE IAAATVAGAL SLEDAAVVA	LRAGLIGRYL	

Fig 4B

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	751		800
niddam...	HG.GAMLSVQ	AAEHDLDQLA	HTHG..VEIA AVNGPTHCVL SGPRTALEET
platenol.	VG.GGMWSVG	ASESVVRGVV	EGLGEWVSVA AVNGPRSVVL SGDVGVLESV
monensin	AP.GAMAAWQ	ATADEAAEQL	AGHERHVTVA AVNGPDSVVV SGDRATVDEL
oleandom	GG.GVMLSQV	APESVAPLL	LGREAHVGLA AVNGPDAVVV SGERGHVAAI
tylosin	AGRGAMAAVP	LPAGEVEAGL	.AKWPGVEVA AVNGPASTVV SGDRRAVAGY
	801		850
niddam...	AQHLREQNVR	HTWLKVSHAF	HSALMDPMLG AFRDTLNTLN Y..QPPTIPL
platenol.	VASLMGDGVE	YRRLDVSHGF	HSVLMPEVLG EFRGVVESLE FGRVPRGVVV
monensin	TAAWRGRGRK	AHHLKVSHAF	HSPHMDPILD ELRAVAAGLT FHE..PVIPV
oleandom	EQILRDRGRK	SRYLRVSHAF	HSPLMEPVLE EFAEAVAGLT FRA..PTTPL
tylosin	VAVCQAEQVQ	ARLIPVDYAS	HSRHVEDLKG ELERVLSGI. .RPRSPRVPV
	851		900
niddam...	ISNLTGQIA.DPNHL	CTPDYWIDHA RHTVRFADAV QTAHHQGT
platenol.	VSGVSGGVV.GSGEL	GDPGYWVRHA REAVRFADGV GVVRGLGVGT
monensin	VSNVTGELVT	ATATGSGAGQ	ADPEYWARHA REPVRFLSGV RGLCERGVTT
oleandom	VSNLTG....	..APVDDRTM	ATPAYWVRHV REAVRFGDGI RALGKLGTS
tylosin	CSTVAGEQPG	EPVF.....	.DAGYWFRNL RNRVEFSAVV GGLLEEGHRR
	901		950
niddam...	YLEIGPHPTL	TTLHHTL..	.DNP..... T TIPTLHRERP
platenol.	LVEVGPHGVL	TGMAGECLGA	GDDV..... V VVPAMRRGRA
monensin	FVELGPDAPL	SAMARDCFPA	P..... .ADRSRPRPA AIATCRGRD
oleandom	FLEVGPDGVL	TAMARACVTA	APEPGHRGEQ GADADAHTAL LLPALRRGRD
tylosin	FIEVSAHPVL	V.....HAIEQ TAEAADRSVH ATGTLRRQDD
	951		
niddam...	EPETLTQAIA	AVGVRTDGID	WAVLCGASRP RRVELPTYAF
platenol.	EREVFEEALA	TVFTRDAGLD	ATALHTGSTG RRIDLPTTPF
monensin	EVATFLRSLA	QAYVRGADV	FTRAYGATAT RRFPLPTYPF
oleandom	EARSLTEAVA	RLHLHGVPMD	WTSVLGGDVS .RVPLPTYAF
tylosin	SPHRLLTSTA	EAWAHGATLT	WDPAL..PPG HLTTLPTYPF

niddam: niddamycin; platenol: platenolide I (spiramycin); oleandom: oleandomycin.

FIG. 4C

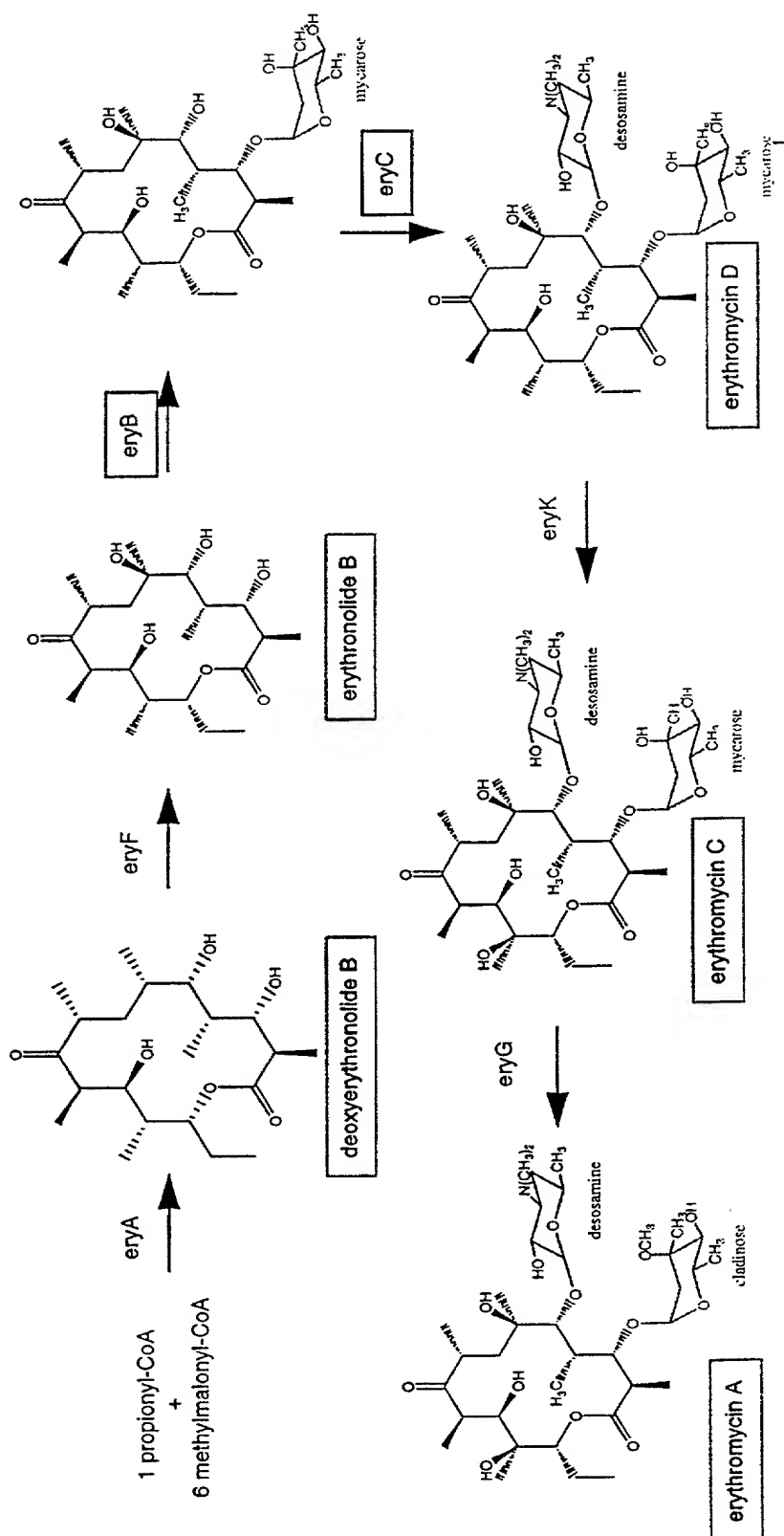
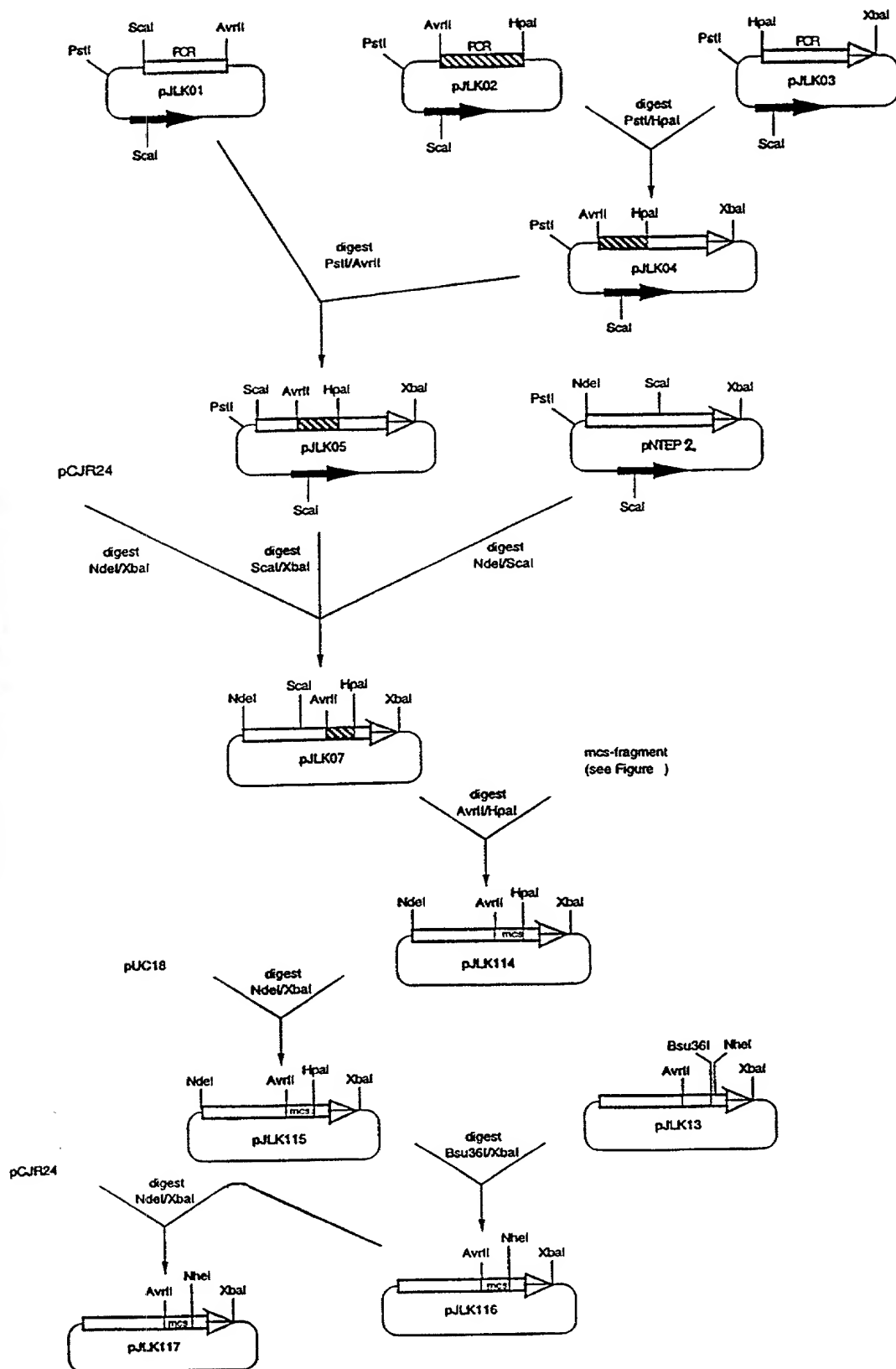


Fig. 5



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Figure 7

forward (Plf):

5'-CTA GGC CGG GCC GGA CTG GTA GAT CTG CCT ACG TAT CCT TTC CAG GGC AAG CGG TTC TGG CTG CAG CCG GAC CGC ACT AGT CCT CGT GAC GAG
GGA GAT GCA TCG AGC CTG AGG GAC CGG TT-3'

backward (Plb):

5'-AAC CGG TCC CTC AGG CTC GAT GCA TCT CCC TCG TCA CGA GGA CTA GTG CGG TCC GGC TGC AGC CAG AAC CGC TTG CCC TGG AAA GGA TAC GTA
GGC AGA TCT ACC AGT CCG GCC CGG C-3'

oligos annealed:

CTAGGCCGGCCGGACTGTGATCTGCCTACGTATCCTTCCAGGGCAAGCGGTTCTGGCTGCAGCCCGGACCCGACTAGTCTCGTGACGAGGGAGATGCATCGAGCCTGAGGGACCGGTT
CGGCCCGCCCTGACCATCTAGACGGATGCATAGGAAGGTCCCGTTCCGCCAAGACCCGACGTCGGCCCTGGCGTGATCAGGAGCACTGCTCCCTCTACGTAGCTCGGACTCCCTGGCCAA

AvrII BglII SnaBI PstI SpeI NsiI Bsu36I HpaI

STATEMENT OF ATTORNEY AND POWER TO INSPECT

As a below named inventor, I hereby declare:

that my residence, post office address and citizenship are as stated below next to my name;

that I verily believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the invention entitled: **POLYKETIDES AND THEIR SYNTHESIS**, the specification of which [check one(s) applicable]

- ☒ was filed 29 June 1999 as International Patent Application Serial No. PCT/GB99/02042, on which U.S. National Stage Application Serial No. 09/720,841 is based; and/or
- ☐ was amended by Amendment filed _____ (if applicable); and/or
- ☐ is attached to this Declaration, Power of Attorney and Power to Inspect;

that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above; and

that I acknowledge my duty to disclose information which is material to the examination of this application in accordance with Rule 56(a) [37 C.F.R. §1.56(a)].

CLAIM UNDER 35 U.S.C. §119: I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application of which priority is claimed:

Prior Foreign Application(s) Appln No.	Country	Filing Date Day-Mon-Year	Priority Claimed Yes - No
9814006.4	Great Britain	29 June 1998	Yes

POWER OF ATTORNEY: As inventor, I hereby appoint **DANN, DORFMAN, HERRELL AND SKILLMAN, P.C.** of Philadelphia, Pennsylvania, and the following individual(s) as my attorneys or agents with full power of substitution to prosecute this application and to transact all business in the United States Patent and Trademark Office connected therewith: **Patrick J. Hagan, Reg. No. 27,643** and **Kathleen D. Nigaut, Ph.D., Reg. 43,047**.

POWER TO INSPECT: I hereby give **DANN, DORFMAN, HERRELL AND SKILLMAN, P.C.** of Philadelphia, Pennsylvania or its duly accredited representatives power to inspect and obtain copies of the papers on file relating to this application.

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Facsimile: (215) 563-4044

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Signature [Signature]

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Signature Hamish Alastair Irvine
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<120> Polyketides and their synthesis

<130> IS/CP5787585

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20

25

30

Arg Arg Phe Ala Asp Asp Gly Arg Leu Gly Arg Leu Ala Gly Glu Val

35

40

45

09/720841 PCT/PTO

Ser Asp Phe Val Pro Glu Asp His Leu Pro Lys Arg Leu Leu Val Gln
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Thr Asp Pro Met Thr Gln Met Thr Ala Leu Ala Ala Glu Trp Ala
65 70 75 80

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Ile Thr Ala Ser Ala Ser Gly Gly Phe Ala Ser Gly Gln Arg Glu Leu
100 105 110

Gln Asn Leu Trp Ser Lys Gly Pro Ala His Val Ser Ala Tyr Met Ser
115 120 125

Phe Ala Trp Phe Tyr Ala Val Asn Thr Gly Gln Ile Ala Ile Arg His
130 135 140

Asp Leu Arg Gly Pro Val Gly Val Val Val Ala Glu Gln Ala Gly Gly
145 150 155 160

Leu Asp Ala Leu Ala His Ala Arg Arg Lys Val Arg Gly Gly Ala Glu
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Leu Ile Val Ser Gly Ala Met Asp Ser Ser Leu Cys Pro Tyr Gly Met
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Ala Ala Gln Val Arg Ser Gly Arg Leu Ser Gly Ser Asp Asp Pro Thr
195 200 205

Ala Gly Tyr Leu Pro Phe Asp Arg Arg Ala Ala Gly His Val Pro Gly
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Glu Gly Gly Ala Ile Leu Ala Val Glu Asp Ala Glu Arg Val Ala Glu
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Arg Gly Gly Lys Val Tyr Gly Ser Ile Ala Gly Thr Ala Ser Phe Asp
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FBI LABORATORY

Pro Pro Pro Gly Ser Gly Arg Pro Ser Ala Leu Ala Arg Ala Val Glu
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Thr Ala Leu Ala Asp Ala Gly Leu Asp Arg Ser Asp Ile Ala Val Val
 275 280 285

Phe Ala Asp Gly Ala Ala Val Gly Glu Leu Asp Val Ala Glu Ala Glu
 290 295 300

Ala Leu Ala Ser Val Phe Gly Pro His Arg Val Pro Val Thr Val Pro
 305 310 315 320

Lys Thr Leu Thr Gly Arg Leu Tyr Ser Gly Ala Gly Pro Leu Asp Val
 325 330 335

Ala Thr Gly Leu Leu Ala Leu Arg Asp Glu Val Val Pro Ala Thr Gly
 340 345 350

His Val His Pro Asp Pro Asp Leu Pro Leu Asp Val Val Thr Gly Arg
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Asp Gly Arg Leu Gly Arg Leu Ala Gly Glu Val Ser Asp Phe Val Pro
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Glu Asp His Leu Pro Lys Arg Leu Leu Ala Gln Thr Asp Pro Met Thr
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Gln Tyr Ala Leu Ala Ala Ala Glu Trp Ala Leu Arg Glu Ser Gly Cys
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Gly Val Val Val Ala Glu Gln Ala Gly Gly Leu Asp Ala Leu Ala His
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Ala Arg Arg Lys Val Arg Gly Gly Ala Glu Leu Ile Val Ser Gly Ala
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 35 40 45

Gly Gln Ile Asp Asp Phe His Ala Pro Asp His Ile Pro Gly Arg Leu
 50 55 60

Leu Pro Gln Thr Asp Pro Ser Thr Arg Leu Ala Leu Thr Ala Ala Asp
 65 70 75 80

Trp Ala Leu Gln Asp Ala Lys Ala Asp Pro Glu Ser Leu Thr Asp Tyr
 85 90 95

Asp Met Gly Val Val Thr Ala Asn Ala Cys Gly Gly Phe Asp Phe Thr
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His Arg Glu Phe Arg Lys Leu Trp Ser Glu Gly Pro Lys Ser Val Ser
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Ile Asp Leu Val Leu Gly Glu Pro Arg Ser Thr Ala Pro Arg Thr Ala
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Arg Arg Phe Ala Pro Thr Pro
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Gly Gln Val Leu Asp Phe Asp Ala Thr Glu His Leu Pro Lys Arg Leu
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0073044 133 430260

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Ser Ile Arg His Gly Leu Arg Gly Pro Gly Ser Val Leu Val Ala Glu
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Ala Thr Phe Asp Pro Ala Pro Gly Ser Glu Arg Pro Pro Ala Leu Arg
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Arg Ala Ile Glu Leu Ala Leu Ala Asp Ala Glu Leu Arg Pro Glu Gln
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Val Asp Val Val Phe Ala Asp Ala Ala Gly Val Ala Glu Leu Asp Ala
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Ile Glu Ala Ala Ala Ile Arg Glu Leu Phe Gly Pro Ser Gly Val Pro
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097004-1430260

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Tyr Pro Ser Arg Leu Ala Gly Gln Ile Asp Asp Phe Glu Ala Ser Glu
50 55 60

His Leu Pro Ser Arg Leu Leu Pro Gln Thr Asp Val Ser Thr Arg Tyr
65 70 75 80

Ala Leu Ala Ala Ala Asp Trp Ala Leu Ala Asp Ala Gly Val Gly Pro
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Glu Ser Gly Leu Asp Asp Tyr Asp Leu Gly Val Val Thr Ser Thr Ala
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Gln Gly Gly Phe Asp Phe Thr His Arg Glu Phe His Lys Leu Trp Ser
115 120 125

Gln Gly Pro Ala Tyr Val Ser Val Tyr Glu Ser Phe Ala Trp Phe Tyr
130 135 140

Ala Val Asn Thr Gly Gln Ile Ser Ile Arg Asn Thr Met Arg Gly Pro
145 150 155 160

Ser Ala Ala Leu Val Gly Glu Gln Ala Gly Gly Leu Asp Ala Ile Gly
165 170 175

His Ala Arg Arg Thr Val Arg Arg Gly Pro Gly Trp Cys Ser Ala Val
180 185 190

Ala Ser Thr Arg Arg Ser Thr Arg Gly Ala Ser Ser Ser Gln Leu Ser
195 200 205

Gly Gly Leu Val Ser Thr Val Ala Asp Pro Glu Arg Ala Tyr Leu Pro
210 215 220

Phe Asp Val Asp Ala Ser Gly Tyr Val Pro Gly Glu Gly Gly Ala Val
225 230 235 240

Leu Ile Val Glu Asp Ala Asp Ser Ala Arg Ala Arg Gly Ala Glu Arg
245 250 255

Ile Tyr Val Arg Ser Pro Leu Arg Arg Asp Pro Ala Pro Gly Ser Gly
260 265 270

Arg Pro Pro Ala Leu Gly Arg Ala Ala Glu Leu Ala Leu Ala Glu Ala
 275 280 285

Gly Leu Thr Pro Ala Asp Ile Ser Val Val Phe Ala Asp Gly Ala Gly
 290 295 300

Val Pro Glu Leu Asp Arg Ala Glu Ala Asp Thr Leu Ala Arg Leu Phe
 305 310 315 320

Gly Pro Arg Gly Val Pro Val Thr Ala Pro Lys Ala Leu Thr Gly Arg
 325 330 335

Leu Cys Ala Gly Gly Gly Pro Ala Asp Leu Ala Ala Ala Leu Leu Ala
 340 345 350

Leu Arg Asp Gln Val Ile Pro Ala Thr Gly Arg His Arg Ala Val Pro
 355 360 365

Asp Ala Tyr Ala Leu Asp Leu Val Thr Gly Arg Pro Arg Glu Ala Ala
 370 375 380

Leu Ser Ala Ala Leu Val Leu Ala Arg Gly Arg His Gly Phe Asn Ser
 385 390 395 400

Ala Val Val Val Thr Leu Arg Gly Ser Asp His Arg Arg Pro Thr
 405 410 415

<210> 6

<211> 409

<212> PRT

<213> Streptomyces nogalater

<400> 6

Met Thr Ala Ala Val Val Val Thr Gly Leu Gly Val Val Ala Pro Thr
 1 5 10 15

Gly Leu Gly Val Arg Glu His Trp Ser Ser Thr Val Arg Gly Ala Ser
20 25 30

Ala Ile Gly Pro Val Thr Arg Phe Asp Ala Gly Arg Tyr Pro Ser Lys
35 40 45

Leu Ala Gly Glu Val Pro Gly Phe Val Pro Glu Asp His Leu Pro Ser
50 55 60

Arg Leu Met Pro Gln Thr Asp His Met Thr Arg Leu Ala Leu Val Ala
65 70 75 80

Ala Asp Trp Ala Phe Gln Asp Ala Ala Val Asp Pro Ser Lys Leu Pro
85 90 95

Glu Tyr Gly Val Gly Val Val Thr Ala Ser Ser Ala Gly Gly Phe Glu
100 105 110

Phe Gly His Arg Glu Leu Gln Asn Leu Trp Ser Leu Gly Pro Gln Tyr
115 120 125

Val Ser Ala Tyr Gln Ser Phe Ala Trp Phe Tyr Ala Val Asn Thr Gly
130 135 140

Gln Val Ser Ile Arg His Gly Leu Arg Gly Pro Gly Gly Val Leu Val
145 150 155 160

Thr Glu Gln Ala Gly Gly Leu Asp Ala Leu Gly Gln Ala Arg Arg Gln
165 170 175

Leu Arg Arg Gly Leu Pro Met Val Val Ala Gly Ala Val Asp Gly Ser
180 185 190

Pro Cys Pro Trp Gly Trp Val Ala Gln Leu Ser Ser Gly Gly Leu Ser
195 200 205

Thr Ser Asp Asp Pro Arg Arg Ala Tyr Leu Pro Phe Asp Ala Ala Ala
210 215 220

0973044 1930

Gly Gly His Val Pro Gly Glu Gly Gly Ala Leu Leu Val Leu Glu Ser
225 230 235 240

Asp Glu Ser Ala Arg Ala Arg Gly Val Thr Arg Trp Tyr Gly Arg Ile
245 250 255

Asp Gly Tyr Ala Ala Thr Phe Asp Pro Pro Pro Gly Ser Gly Arg Pro
260 265 270

Pro Asn Leu Leu Arg Ala Ala Gln Ala Ala Leu Asp Asp Ala Glu Val
275 280 285

Gly Pro Glu Ala Val Asp Val Val Phe Ala Asp Ala Ser Gly Thr Pro
290 295 300

Asp Glu Asp Ala Ala Glu Ala Asp Ala Val Arg Arg Leu Phe Gly Pro
305 310 315 320

Tyr Gly Val Pro Val Thr Ala Pro Lys Thr Met Thr Gly Arg Leu Ser
325 330 335

Ala Gly Gly Ala Ala Leu Asp Val Ala Thr Ala Leu Leu Ala Leu Arg
340 345 350

Glu Gly Val Val Pro Pro Thr Val Asn Val Ser Arg Pro Arg Pro Glu
355 360 365

Tyr Glu Leu Asp Leu Val Leu Ala Pro Arg Arg Thr Pro Leu Ala Arg
370 375 380

Ala Leu Val Leu Ala Arg Gly Arg Gly Gly Phe Asn Ala Ala Met Val
385 390 395 400

Val Ala Gly Pro Arg Ala Glu Thr Arg
405

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<210> 7

<211> 409

<212> PRT

<213> *Streptomyces glaucescens*

<400> 7

Met Ser Ala Pro Ala Pro Val Val Val Thr Gly Leu Gly Ile Val Ala
 1 5 10 15

Pro Asn Gly Thr Gly Thr Glu Glu Tyr Trp Ala Ala Thr Leu Ala Gly
 20 25 30

Lys Ser Gly Ile Asp Val Ile Gln Arg Phe Asp Pro His Gly Tyr Pro
 35 40 45

Val Arg Val Gly Gly Glu Val Leu Ala Phe Asp Ala Ala Ala His Leu
 50 55 60

Pro Gly Arg Leu Leu Pro Gln Thr Asp Arg Met Thr Gln His Ala Leu
 65 70 75 80

Val Ala Ala Glu Trp Ala Leu Ala Asp Ala Gly Leu Glu Pro Glu Lys
 85 90 95

Gln Asp Glu Tyr Gly Leu Gly Val Leu Thr Ala Ala Gly Ala Gly Gly
 100 105 110

Phe Glu Phe Gly Gln Arg Glu Met Gln Lys Leu Trp Gly Thr Gly Pro
 115 120 125

Glu Arg Val Ser Ala Tyr Gln Ser Phe Ala Trp Phe Tyr Ala Val Asn
 130 135 140

Thr Gly Gln Ile Ser Ile Arg His Gly Met Arg Gly His Ser Ser Val
 145 150 155 160

Phe Val Thr Glu Gln Ala Gly Gly Leu Asp Ala Ala Ala His Ala Ala
 165 170 175

0970044 001304

Arg Leu Leu Arg Lys Gly Thr Leu Asn Thr Ala Leu Thr Gly Gly Cys
 180 185 190

Glu Ala Ser Leu Cys Pro Trp Gly Leu Val Ala Gln Ile Pro Ser Gly
 195 200 205

Phe Leu Ser Glu Ala Thr Asp Pro His Asp Ala Tyr Leu Pro Phe Asp
 210 215 220

Ala Arg Ala Ala Gly Tyr Val Pro Gly Glu Gly Gly Ala Met Leu Val
 225 230 235 240

Ala Glu Arg Ala Asp Ser Ala Arg Glu Arg Asp Ala Ala Thr Val Tyr
 245 250 255

Gly Arg Ile Ala Gly His Ala Ser Thr Phe Asp Ala Arg Pro Gly Thr
 260 265 270

Gly Arg Pro Thr Gly Pro Ala Arg Ala Ile Arg Leu Ala Leu Glu Glu
 275 280 285

Ala Arg Val Ala Pro Glu Asp Val Asp Val Val Tyr Ala Asp Ala Ala
 290 295 300

Gly Val Pro Ala Leu Asp Arg Ala Glu Ala Glu Ala Leu Ala Glu Val
 305 310 315 320

Phe Gly Pro Gly Ala Val Pro Val Thr Ala Pro Lys Thr Met Thr Gly
 325 330 335

Arg Leu Tyr Ala Gly Gly Ala Ala Leu Asp Val Ala Thr Ala Leu Leu
 340 345 350

Ser Ile Arg Asp Cys Val Val Pro Pro Thr Val Gly Thr Gly Ala Pro
 355 360 365

Ala Pro Gly Leu Gly Ile Asp Leu Val Leu His Gln Pro Arg Glu Leu
 370 375 380

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Arg Val Asp Thr Ala Leu Val Val Ala Arg Gly Met Gly Gly Phe Asn
 385 390 395 400

Ser Ala Leu Val Val Arg Arg His Gly
 405

<210> 8

<211> 402

<212> PRT

<213> *Streptomyces cinnamonensis*

<400> 8

Met Thr Pro Val Ala Val Thr Gly Met Gly Ile Ala Ala Pro Asn Gly
 1 5 10 15

Leu Gly Arg Pro Thr Thr Gly Arg Pro Pro Trp Ala Pro Arg Ala Ala
 20 25 30

Ser Ala Ala Ser Thr Arg Phe Asp Pro Ser Gly Tyr Pro Ala Gln Leu
 35 40 45

Ala Gly Glu Ile Pro Gly Phe Arg Ala Ala Glu His Leu Pro Gly Arg
 50 55 60

Leu Val Pro Gln Thr Asp Arg Val Thr Arg Leu Ser Leu Ala Ala Ala
 65 70 75 80

Asp Trp Ala Leu Ala Asp Ala Gly Val Glu Val Ala Ala Phe Asp Pro
 85 90 95

Leu Asp Met Gly Val Val Thr Ala Ser His Ala Gly Gly Phe Glu Phe
 100 105 110

Gly Gln Asp Glu Leu Gln Lys Leu Leu Gly Gln Gly Gln Pro Val Leu
 115 120 125

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Ala Pro Leu Asp Val Ala Cys Ala Leu Leu Ala Met Arg Ala Gly Val
 340 345 350

Ile Pro Pro Thr Val His Ile Asp Pro Cys Pro Glu Tyr Asp Leu Asp
 355 360 365

Leu Val Leu Tyr Gln Val Arg Pro Ala Ala Leu Arg Thr Ala Leu Gly
 370 375 380

Gly Ala Arg Gly His Gly Gly Phe Asn Ser Ala Leu Val Val Arg Ala
 385 390 395 400

Gly Gln

<210> 9

<211> 404

<212> PRT

<213> Streptomyces venezuelae

<400> 9

Met Ser Ala Ser Val Val Val Thr Gly Leu Gly Val Ala Ala Pro Asn
 1 5 10 15

Gly Leu Gly Arg Glu Asp Phe Trp Ala Ser Thr Leu Gly Gly Lys Ser
 20 25 30

Gly Ile Gly Pro Leu Thr Arg Phe Asp Pro Thr Gly Tyr Pro Ala Arg
 35 40 45

Leu Ala Gly Glu Val Pro Gly Phe Ala Ala Glu Glu His Leu Pro Ser
 50 55 60

Arg Leu Leu Pro Gln Thr Asp Arg Met Thr Arg Leu Ala Leu Val Ala
 65 70 75 80

0970041-094304

Pro Gly Asp Ile Asp Val Val Phe Ala Asp Ala Ala Ala Val Pro Glu
290 295 300

Leu Asp Arg Val Glu Ala Glu Ala Leu Asn Ala Val Phe Gly Thr Gly
305 310 315 320

Ala Val Pro Val Thr Ala Pro Lys Thr Met Thr Gly Arg Leu Tyr Ser
325 330 335

Gly Ala Ala Pro Leu Asp Leu Ala Ala Ala Phe Leu Ala Met Asp Glu
340 345 350

Gly Val Ile Pro Pro Thr Val Asn Val Glu Pro Asp Ala Ala Tyr Gly
355 360 365

Leu Asp Leu Val Val Gly Gly Pro Arg Thr Ala Glu Val Asn Thr Ala
370 375 380

Leu Val Ile Ala Arg Gly His Gly Gly Phe Asn Ser Ala Met Val Val
385 390 395 400

Arg Ser Ala Asn

<210> 10

<211> 424

<212> PRT

<213> Streptomyces coelicolor

<400> 10

Met Ser Gly Pro Gln Arg Thr Gly Thr Gly Gly Gly Ser Arg Arg Ala
1 5 10 15

Val Val Thr Gly Leu Gly Val Leu Ser Pro His Gly Thr Gly Val Glu
20 25 30

0973044 04304

Ala His Trp Lys Ala Val Ala Asp Gly Thr Ser Ser Leu Gly Pro Val
35 40 45

Thr Arg Glu Gly Cys Ala His Leu Pro Leu Arg Val Ala Gly Glu Val
50 55 60

His Gly Phe Asp Ala Ala Glu Thr Val Glu Asp Arg Phe Leu Val Gln
65 70 75 80

Thr Asp Arg Phe Thr His Phe Ala Leu Ser Ala Thr Gln His Ala Leu
85 90 95

Ala Asp Ala Arg Phe Gly Arg Ala Asp Val Asp Ser Pro Tyr Ser Val
100 105 110

Gly Val Val Thr Ala Ala Gly Ser Gly Gly Gly Glu Phe Gly Gln Arg
115 120 125

Glu Leu Gln Asn Leu Trp Gly His Gly Ser Arg His Val Gly Pro Tyr
130 135 140

Gln Ser Ile Ala Trp Phe Tyr Ala Ala Ser Thr Gly Gln Val Ser Ile
145 150 155 160

Arg Asn Asp Phe Lys Gly Pro Cys Gly Val Val Ala Ala Asp Glu Ala
165 170 175

Gly Gly Leu Asp Ala Leu Ala His Ala Ala Leu Ala Val Arg Asn Gly
180 185 190

Thr Asp Thr Val Val Cys Gly Ala Thr Glu Ala Pro Leu Ala Pro Tyr
195 200 205

Ser Ile Val Cys Gln Leu Gly Tyr Pro Glu Leu Ser Arg Ala Thr Glu
210 215 220

Pro Asp Arg Ala Tyr Arg Pro Phe Thr Glu Ala Ala Cys Gly Phe Ala
225 230 235 240

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<210> 11

<211> 421

<212> PRT

<213> Streptomyces violaceoruber

<400> 11

Met Thr Arg Arg Val Val Ile Thr Gly Val Gly Val Arg Ala Pro Gly
 1 5 10 15

Gly Ser Gly Thr Lys Glu Phe Trp Asp Leu Leu Thr Ala Gly Arg Thr
 20 25 30

Ala Thr Arg Pro Ile Ser Phe Phe Asp Ala Ser Pro Phe Arg Ser Arg
 35 40 45

Ile Ala Gly Glu Ile Asp Phe Asp Ala Val Ala Glu Gly Phe Ser Pro
 50 55 60

Arg Glu Val Arg Arg Met Asp Arg Ala Thr Gln Phe Ala Val Ala Cys
 65 70 75 80

Thr Arg Asp Ala Leu Ala Asp Ser Gly Leu Asp Thr Gly Ala Leu Asp
 85 90 95

Pro Ser Arg Ile Gly Val Ala Leu Gly Ser Ala Val Ala Ser Ala Thr
 100 105 110

Ser Leu Glu Asn Glu Tyr Leu Val Met Ser Asp Ser Gly Arg Glu Trp
 115 120 125

Leu Val Asp Pro Ala His Leu Ser Pro Met Met Phe Asp Tyr Leu Ser
 130 135 140

Pro Gly Val Met Pro Ala Glu Val Ala Trp Ala Ala Gly Ala Glu Gly
 145 150 155 160

Pro Val Thr Met Val Ser Asp Gly Cys Thr Ser Gly Leu Asp Ser Val
 165 170 175

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Gly Tyr Ala Val Gln Gly Thr Arg Glu Gly Ser Ala Asp Val Val Val
180 185 190

Ala Gly Ala Ala Asp Thr Pro Val Ser Pro Ile Val Val Ala Cys Phe
195 200 205

Asp Ala Ile Lys Ala Thr Thr Pro Arg Asn Asp Asp Pro Ala His Ala
210 215 220

Ser Arg Pro Phe Asp Gly Thr Arg Asn Gly Phe Val Leu Ala Glu Gly
225 230 235 240

Ala Ala Met Phe Val Leu Glu Glu Tyr Glu Ala Ala Gln Arg Arg Gly
245 250 255

Ala His Ile Tyr Ala Glu Val Gly Gly Tyr Ala Thr Arg Ser Gln Ala
260 265 270

Tyr His Met Thr Gly Leu Lys Lys Asp Gly Arg Glu Met Ala Glu Ser
275 280 285

Ile Arg Ala Ala Leu Asp Glu Ala Arg Leu Asp Arg Thr Ala Val Asp
290 295 300

Tyr Val Asn Ala His Gly Ser Gly Thr Lys Gln Asn Asp Arg His Glu
305 310 315 320

Thr Ala Ala Phe Lys Arg Ser Leu Gly Glu His Ala Tyr Ala Val Pro
325 330 335

Val Ser Ser Ile Lys Ser Met Gly Gly His Ser Leu Gly Ala Ile Gly
340 345 350

Ser Ile Glu Ile Ala Ala Ser Val Leu Ala Ile Glu His Asn Val Val
355 360 365

Pro Pro Thr Ala Asn Leu His Thr Pro Asp Pro Glu Cys Asp Leu Asp
370 375 380

097200844 097200844

Tyr Val Pro Leu Thr Ala Arg Glu Gln Arg Val Asp Thr Val Leu Thr
 385 390 395 400

Val Gly Ser Gly Phe Gly Gly Phe Gln Ser Ala Met Val Leu His Arg
 405 410 415

Pro Glu Glu Ala Ala
 420

<210> 12

<211> 422

<212> PRT

<213> *Saccharopolyspora hirsuta*

<400> 12

Met Thr Arg Arg Val Val Ile Thr Gly Val Gly Val Arg Ala Pro Gly
 1 5 10 15

Gly Leu Gly Ala Lys Asn Phe Trp Glu Leu Leu Thr Ser Gly Arg Thr
 20 25 30

Ala Thr Arg Arg Ile Ser Phe Phe Asp Pro Thr Pro Asn Arg Ser Gln
 35 40 45

Ile Ala Ala Glu Cys Asp Phe Asp Pro Glu His Glu Gly Leu Ser Pro
 50 55 60

Arg Glu Ile Arg Arg Met Asp Arg Ala Ala Gln Phe Ala Val Val Cys
 65 70 75 80

Thr Arg Asp Ala Val Ala Asp Ser Gly Leu Glu Phe Glu Gln Val Pro
 85 90 95

Pro Glu Arg Ile Gly Val Ser Leu Gly Ser Ala Val Ala Ala Ala Thr
 100 105 110

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Thr Ala Ala Phe Lys Arg Ser Leu Gly Glu His Ala Tyr Arg Thr Pro
 325 330 335

Val Ser Ser Ile Lys Ser Met Val Gly His Ser Leu Gly Ala Ile Gly
 340 345 350

Ser Ile Glu Val Ala Ala Cys Ala Leu Ala Ile Glu His Gly Val Val
 355 360 365

Pro Pro Thr Ala Asn Leu His Glu Pro Asp Pro Glu Cys Asp Leu Asp
 370 375 380

Tyr Val Pro Leu Thr Ala Arg Glu Gln Arg Val Asp Thr Val Leu Ser
 385 390 395 400

Val Gly Ser Gly Phe Gly Gly Phe Gln Ser Ala Met Val Leu Arg Arg
 405 410 415

Leu Gly Gly Ala Asn Ser
 420

<210> 13

<211> 424

<212> PRT

<213> Streptomyces coelicolor

<400> 13

Met Lys Arg Arg Val Val Ile Thr Gly Val Gly Val Arg Ala Pro Gly
 1 5 10 15

Gly Asn Gly Thr Arg Gln Phe Trp Glu Leu Leu Thr Ser Gly Arg Thr
 20 25 30

Ala Thr Arg Arg Ile Ser Phe Phe Asp Pro Ser Pro Tyr Arg Ser Gln
 35 40 45

Val Ala Ala Glu Ala Asp Phe Asp Pro Val Ala Glu Gly Phe Gly Pro
50 55 60

Arg Glu Leu Asp Arg Met Asp Arg Ala Ser Gln Phe Ala Val Ala Cys
65 70 75 80

Ala Arg Glu Ala Phe Ala Ala Ser Gly Leu Asp Pro Asp Thr Leu Asp
85 90 95

Pro Ala Arg Val Gly Val Ser Leu Gly Ser Ala Val Ala Ala Ala Thr
100 105 110

Ser Leu Glu Arg Glu Tyr Leu Leu Leu Ser Asp Ser Gly Arg Asp Trp
115 120 125

Glu Val Asp Ala Ala Trp Leu Ser Arg His Met Phe Asp Tyr Leu Val
130 135 140

Pro Ser Val Met Pro Ala Glu Val Ala Trp Ala Val Gly Ala Glu Gly
145 150 155 160

Pro Val Thr Met Val Ser Thr Gly Cys Thr Ser Gly Leu Asp Ser Val
165 170 175

Gly Asn Ala Val Arg Ala Ile Glu Glu Gly Ser Ala Asp Val Met Phe
180 185 190

Ala Gly Ala Ala Asp Thr Pro Ile Thr Pro Ile Val Val Ala Cys Phe
195 200 205

Asp Ala Ile Arg Ala Thr Thr Ala Arg Asn Asp Asp Pro Glu His Ala
210 215 220

Ser Arg Pro Phe Asp Gly Thr Arg Asp Gly Phe Val Leu Ala Glu Gly
225 230 235 240

Ala Ala Met Phe Val Leu Glu Asp Tyr Asp Ser Ala Leu Ala Arg Gly
245 250 255

119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000

Ala Arg Ile His Ala Glu Ile Ser Gly Tyr Ala Thr Arg Cys Asn Ala
 260 265 270

Tyr His Met Thr Gly Leu Lys Ala Asp Gly Arg Glu Met Ala Glu Thr
 275 280 285

Ile Arg Val Ala Leu Asp Glu Ser Arg Thr Asp Ala Thr Asp Ile Asp
 290 295 300

Tyr Ile Asn Ala His Gly Ser Gly Thr Arg Gln Asn Asp Arg His Glu
 305 310 315 320

Thr Ala Ala Tyr Lys Arg Ala Leu Gly Glu His Ala Arg Arg Thr Pro
 325 330 335

Val Ser Ser Ile Lys Ser Met Val Gly His Ser Leu Gly Ala Ile Gly
 340 345 350

Ser Leu Glu Ile Ala Ala Cys Val Leu Ala Leu Glu His Gly Val Val
 355 360 365

Pro Pro Thr Ala Asn Leu Arg Thr Ser Asp Pro Glu Cys Asp Leu Asp
 370 375 380

Tyr Val Pro Leu Glu Ala Arg Glu Arg Lys Leu Arg Ser Val Leu Thr
 385 390 395 400

Val Gly Ser Gly Phe Gly Gly Phe Gln Ser Ala Met Val Leu Arg Asp
 405 410 415

Ala Glu Thr Ala Gly Ala Ala Ala
 420

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<210> 14

<211> 420

<212> PRT

<213> Streptomyces cinnamonensis

<400> 14

Met Thr Gln Arg Arg Val Ala Ile Thr Gly Ile Glu Val Leu Ala Pro
 1 5 10 15

Gly Gly Leu Gly Arg Lys Glu Phe Trp Gln Leu Leu Ser Glu Gly Arg
 20 25 30

Thr Ala Thr Arg Gly Ile Thr Phe Phe Asp Pro Ala Pro Phe Arg Ser
 35 40 45

Lys Val Ala Ala Glu Ala Asp Phe Cys Gly Leu Glu Asn Gly Leu Ser
 50 55 60

Pro Gln Glu Val Arg Arg Met Asp Arg Ala Ala Gln Phe Ala Val Val
 65 70 75 80

Thr Ala Arg Ala Val Glu Asp Ser Gly Ala Glu Leu Ala Ala His Pro
 85 90 95

Pro His Arg Ile Gly Val Val Val Gly Ser Ala Val Gly Ala Thr Met
 100 105 110

Gly Leu Asp Asn Glu Tyr Arg Val Val Ser Asp Gly Gly Arg Leu Asp
 115 120 125

Leu Val Asp His Arg Tyr Ala Val Pro His Leu Tyr Asn Tyr Leu Val
 130 135 140

Pro Ser Ser Phe Ala Ala Glu Val Ala Trp Ala Val Gly Ala Glu Gly
 145 150 155 160

Pro Ser Thr Val Val Ser Thr Gly Cys Thr Ser Gly Ile Asp Ala Val
 165 170 175

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 697044.0330

Gly Leu Asp Glu Glu Tyr Arg Val Val Ser Asp Gly Gly Arg Leu Asp
 115 120 125

Leu Val Asp His Ala Tyr Ala Val Pro His Leu Tyr Asp Tyr Met Val
 130 135 140

Pro Ser Ser Phe Ser Ala Glu Val Ala Trp Ala Val Gly Ala Glu Gly
 145 150 155 160

Pro Asn Thr Val Val Ser Thr Gly Cys Thr Ser Gly Leu Asp Ser Val
 165 170 175

Gly Tyr Ala Arg Gly Glu Leu Ile Arg Glu Gly Ser Ala Asp Val Met
 180 185 190

Ile Ala Gly Ser Ser Asp Ala Pro Ile Ser Pro Ile Thr Met Ala Cys
 195 200 205

Phe Asp Ala Ile Lys Ala Thr Thr Asn Arg Tyr Asp Asp Pro Ala His
 210 215 220

Ala Ser Arg Pro Phe Asp Gly Thr Arg Asn Gly Phe Val Leu Gly Glu
 225 230 235 240

Gly Ala Ala Val Phe Val Leu Glu Glu Leu Glu Ser Ala Arg Ala Arg
 245 250 255

Gly Ala His Ile Tyr Ala Glu Ile Ala Gly Tyr Ala Thr Arg Ser Asn
 260 265 270

Ala Tyr His Met Thr Gly Leu Arg Pro Asp Gly Ala Glu Met Ala Glu
 275 280 285

Ala Ile Arg Val Ala Leu Asp Glu Ala Arg Met Asn Pro Thr Glu Ile
 290 295 300

Asp Tyr Ile Asn Ala His Gly Ser Gly Thr Lys Gln Asn Asp Arg His
 305 310 315 320

Phe Arg Ser Arg Ile Ala Ala Glu Cys Asp Phe Asp Pro Leu Ala Glu
 50 55 60

Gly Leu Thr Pro Gln Gln Ile Arg Arg Met Asp Arg Ala Thr Gln Phe
 65 70 75 80

Ala Val Val Ser Ala Arg Glu Ser Leu Glu Asp Ser Gly Leu Asp Leu
 85 90 95

Gly Ala Leu Asp Ala Ser Arg Thr Gly Val Val Val Gly Ser Ala Val
 100 105 110

Gly Cys Thr Thr Ser Leu Glu Glu Glu Tyr Ala Val Val Ser Asp Ser
 115 120 125

Gly Arg Asn Trp Leu Val Asp Asp Gly Tyr Ala Val Pro His Leu Phe
 130 135 140

Asp Tyr Phe Val Pro Ser Ser Ile Ala Ala Glu Val Ala His Asp Arg
 145 150 155 160

Ile Gly Ala Glu Gly Pro Val Ser Leu Val Ser Thr Gly Cys Thr Ser
 165 170 175

Gly Leu Asp Ala Val Gly Arg Ala Ala Asp Leu Ile Ala Glu Gly Ala
 180 185 190

Ala Asp Val Met Leu Ala Gly Ala Thr Glu Ala Pro Ile Ser Pro Ile
 195 200 205

Thr Val Ala Cys Phe Asp Ala Ile Lys Ala Thr Thr Pro Arg Asn Asp
 210 215 220

Thr Pro Ala Glu Ala Ser Arg Pro Phe Asp Arg Thr Arg Asn Gly Phe
 225 230 235 240

Val Leu Gly Glu Gly Ala Ala Val Phe Val Leu Glu Glu Phe Glu His
 245 250 255

Leu Asp Ala Val Gly Tyr Gly Thr Glu Leu Ile Arg Asp Gly Arg Ala
 180 185 190

Asp Val Val Val Cys Gly Ala Thr Asp Ala Pro Ile Ser Pro Ile Thr
 195 200 205

Val Ala Cys Phe Asp Ala Ile Lys Ala Thr Ser Ala Asn Asn Asp Asp
 210 215 220

Pro Ala His Ala Ser Arg Pro Phe Asp Arg Asn Arg Asp Gly Phe Val
 225 230 235 240

Leu Gly Glu Gly Ser Ala Val Phe Val Leu Glu Glu Leu Ser Ala Ala
 245 250 255

Arg Arg Arg Gly Ala His Ala Tyr Ala Glu Val Arg Gly Phe Ala Thr
 260 265 270

Arg Ser Asn Ala Phe His Met Thr Gly Leu Lys Pro Asp Gly Arg Glu
 275 280 285

Met Ala Glu Ala Ile Thr Ala Ala Leu Asp Gln Ala Arg Arg Thr Gly
 290 295 300

Asp Asp Leu His Tyr Ile Asn Ala His Gly Ser Gly Thr Arg Gln Asn
 305 310 315 320

Asp Arg His Glu Thr Ala Ala Phe Lys Arg Ser Leu Gly Gln Arg Ala
 325 330 335

Tyr Asp Val Pro Val Ser Ser Ile Lys Ser Met Ile Gly His Ser Leu
 340 345 350

Gly Ala Ile Gly Ser Leu Glu Leu Ala Ala Cys Ala Leu Ala Ile Glu
 355 360 365

His Gly Val Ile Pro Pro Thr Ala Asn Tyr Glu Glu Pro Asp Pro Glu
 370 375 380

Lys Leu Glu Ser Glu Tyr Val Ala Leu Ser Ala Gly Gly Ala Asn Trp
 115 120 125

Val Val Asp Pro His Arg Gly Ala Pro Glu Leu Tyr Asp Tyr Phe Val
 130 135 140

Pro Ser Ser Leu Ala Ala Glu Val Ala Trp Leu Ala Gly Ala Glu Gly
 145 150 155 160

Pro Val Asn Ile Val Ser Ala Gly Cys Thr Ser Gly Ile Asp Ser Ile
 165 170 175

Gly Tyr Ala Cys Glu Leu Ile Arg Glu Gly Thr Val Asp Val Met Leu
 180 185 190

Ala Gly Gly Val Asp Ala Pro Ile Ala Pro Ile Thr Val Ala Cys Phe
 195 200 205

Asp Ala Ile Arg Val Thr Ser Asp His Asn Asp Thr Pro Glu Thr Leu
 210 215 220

Ala Pro Phe Ser Arg Ser Arg Asn Gly Phe Val Leu Gly Glu Gly Gly
 225 230 235 240

Ala Ile Val Val Leu Glu Glu Ala Glu Ala Ala Val Arg Arg Gly Ala
 245 250 255

Arg Ile Tyr Ala Glu Ile Gly Gly Tyr Ala Ser Arg Gly Asn Ala Tyr
 260 265 270

His Met Thr Gly Leu Arg Ala Asp Gly Ala Glu Met Ala Ala Ala Ile
 275 280 285

Thr Ala Ala Leu Asp Glu Ala Arg Arg Asp Pro Ser Asp Val Asp Tyr
 290 295 300

Val Asn Ala His Gly Thr Ala Thr Arg Gln Asn Asp Arg His Glu Thr
 305 310 315 320

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Ser Ala Phe Lys Arg Ser Leu Gly Asp His Ala Tyr Arg Val Pro Ile
 325 330 335

Ser Ser Val Lys Ser Met Ile Gly His Ser Leu Gly Ala Ala Gly Ser
 340 345 350

Leu Glu Val Ala Ala Thr Ala Leu Ala Val Glu Tyr Gly Ala Ile Pro
 355 360 365

Pro Thr Ala Asn Leu His Asp Pro Asp Pro Glu Leu Asp Leu Asp Tyr
 370 375 380

Val Pro Leu Thr Ala Arg Glu Lys Arg Val Arg His Ala Leu Thr Val
 385 390 395 400

Gly Ser Gly Phe Gly Gly Phe Gln Ser Ala Met Leu Leu Ser Arg Pro
 405 410 415

Glu Arg

<210> 19

<211> 419

<212> PRT

<213> Streptomyces peucetius

<400> 19

Met Asn Arg Arg Ile Val Ile Thr Gly Ile Gly Val Val Ala Pro Gly
 1 5 10 15

Ala Val Gly Thr Lys Pro Phe Trp Glu Leu Leu Leu Ser Gly Thr Thr
 20 25 30

Ala Thr Arg Ala Ile Ser Thr Phe Asp Ala Thr Pro Phe Arg Ser Arg
 35 40 45

09723041-03201
 19820814-03201

Ile Ala Ala Glu Cys Asp Phe Asp Pro Val Ala Ala Gly Leu Ser Ala
 50 55 60

Glu Gln Ala Arg Arg Leu Asp Arg Ala Gly Gln Phe Ala Leu Val Ala
 65 70 75 80

Gly Gln Glu Ala Leu Ala Asp Ser Gly Leu Arg Ile Asp Glu Asp Ser
 85 90 95

Ala His Arg Val Gly Val Cys Val Gly Thr Ala Val Gly Cys Thr Gln
 100 105 110

Lys Leu Glu Ser Glu Tyr Val Ala Leu Ser Ala Gly Gly Ala His Trp
 115 120 125

Val Val Asp Pro Gly Arg Gly Ser Pro Glu Leu Tyr Asp Tyr Phe Val
 130 135 140

Pro Ser Ser Leu Ala Ala Glu Val Ala Trp Leu Ala Gly Ala Glu Gly
 145 150 155 160

Pro Val Asn Ile Val Ser Ala Gly Cys Thr Ser Gly Ile Asp Ser Ile
 165 170 175

Gly Tyr Ala Cys Glu Leu Ile Arg Glu Gly Thr Val Asp Ala Met Val
 180 185 190

Ala Gly Gly Val Asp Ala Pro Ile Ala Pro Ile Thr Val Ala Cys Phe
 195 200 205

Asp Ala Ile Arg Ala Thr Ser Asp His Asn Asp Thr Pro Glu Thr Ala
 210 215 220

Ser Arg Pro Phe Ser Arg Ser Arg Asn Gly Phe Val Leu Gly Glu Gly
 225 230 235 240

Gly Ala Ile Val Val Leu Glu Glu Ala Glu Ala Ala Val Arg Arg Gly
 245 250 255

47

Asp Tyr Val Pro Arg Glu Ala Arg Glu Arg Thr Leu Arg His Val Leu
385 390 395 400

Ser Val Gly Ser Gly Phe Gly Gly Phe Gln Ser Ala Val Val Leu Ser
405 410 415

Gly Ser Glu Gly Gly Leu Arg
420

<210> 21

<211> 871

<212> PRT

<213> Streptomyces caelestis

<400> 21

Met Ala Gly His Gly Asp Ala Thr Ala Gln Lys Ala Gln Asp Ala Glu
1 5 10 15

Lys Ser Glu Asp Gly Ser Asp Ala Ile Ala Val Ile Gly Met Ser Cys
20 25 30

Arg Phe Pro Gly Ala Pro Gly Thr Ala Glu Phe Trp Gln Leu Leu Ser
35 40 45

Ser Gly Ala Asp Ala Val Val Thr Ala Ala Asp Gly Arg Arg Arg Gly
50 55 60

Thr Ile Asp Ala Pro Ala Asp Phe Asp Ala Ala Phe Phe Gly Met Ser
65 70 75 80

Pro Arg Glu Ala Ala Ala Thr Asp Pro Gln Gln Arg Leu Val Leu Glu
85 90 95

Leu Gly Trp Glu Ala Leu Glu Asp Ala Gly Ile Val Pro Glu Ser Leu
100 105 110

09720841.084301

Arg Gly Glu Ala Ala Ser Val Phe Val Gly Ala Met Asn Asp Asp Tyr
 115 120 125

Ala Thr Leu Leu His Arg Ala Gly Ala Pro Thr Asp Thr Tyr Thr Ala
 130 135 140

Thr Gly Leu Gln His Ser Met Ile Ala Asn Arg Leu Ser Tyr Phe Leu
 145 150 155 160

Gly Leu Arg Gly Pro Ser Leu Val Val Asp Thr Gly Gln Ser Ser Ser
 165 170 175

Leu Val Ala Val Ala Leu Ala Val Glu Ser Leu Arg Gly Gly Thr Ser
 180 185 190

Gly Ile Ala Leu Ala Gly Gly Val Asn Leu Val Leu Ala Glu Glu Gly
 195 200 205

Ser Ala Ala Met Glu Arg Val Gly Ala Leu Ser Pro Asp Gly Arg Cys
 210 215 220

His Thr Phe Asp Ala Arg Ala Asn Gly Tyr Val Arg Gly Glu Gly Gly
 225 230 235 240

Ala Ile Val Val Leu Lys Pro Leu Ala Asp Ala Leu Ala Asp Gly Asp
 245 250 255

Arg Val Tyr Cys Val Val Arg Gly Val Ala Thr Gly Asn Asp Gly Gly
 260 265 270

Gly Pro Gly Leu Thr Val Pro Asp Arg Ala Gly Gln Glu Ala Val Leu
 275 280 285

Arg Ala Ala Cys Asp Gln Ala Gly Val Arg Pro Ala Asp Val Arg Phe
 290 295 300

Val Glu Leu His Gly Thr Gly Thr Pro Ala Gly Asp Pro Val Glu Ala
 305 310 315 320

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Glu Ala Leu Gly Ala Val Tyr Gly Thr Gly Arg Pro Ala Asn Glu Pro
 325 330 335

Leu Leu Val Gly Ser Val Lys Thr Asn Ile Gly His Leu Glu Gly Ala
 340 345 350

Ala Gly Ile Ala Gly Phe Val Lys Ala Ala Leu Cys Leu His Glu Arg
 355 360 365

Ala Leu Pro Ala Ser Leu Asn Phe Glu Thr Pro Asn Pro Ala Ile Pro
 370 375 380

Leu Glu Arg Leu Arg Leu Lys Val Gln Thr Ala His Ala Ala Leu Gln
 385 390 395 400

Pro Gly Thr Gly Gly Gly Pro Leu Leu Ala Gly Val Ser Ala Phe Gly
 405 410 415

Met Gly Gly Thr Asn Cys His Val Val Leu Glu Glu Thr Pro Gly Gly
 420 425 430

Arg Gln Pro Ala Glu Thr Gly Gln Ala Asp Ala Cys Leu Phe Ser Ala
 435 440 445

Ser Pro Met Leu Leu Leu Ser Ala Arg Ser Glu Gln Ala Leu Arg Ala
 450 455 460

Gln Ala Ala Arg Leu Arg Glu His Leu Glu Asp Ser Gly Ala Asp Pro
 465 470 475 480

Leu Asp Ile Ala Tyr Ser Leu Ala Thr Thr Arg Thr Arg Phe Glu His
 485 490 495

Arg Ala Ala Val Pro Cys Gly Asp Pro Asp Arg Leu Ser Ser Ala Leu
 500 505 510

Ala Ala Leu Ala Ala Gly Gln Thr Pro Arg Gly Val Arg Ile Gly Ser
 515 520 525

000004 001111

Thr Asp Ala Asp Gly Arg Leu Ala Leu Leu Phe Thr Gly Gln Gly Ala
 530 535 540

Gln His Pro Gly Met Gly Gln Glu Leu Tyr Thr Thr Asp Pro His Phe
 545 550 555 560

Ala Ala Ala Leu Asp Glu Val Cys Glu Glu Leu Gln Arg Cys Gly Thr
 565 570 575

Gln Asn Leu Arg Glu Val Met Phe Thr Pro Asp Gln Pro Asp Leu Leu
 580 585 590

Asp Arg Thr Glu Tyr Thr Gln Pro Ala Leu Phe Ala Leu Gln Thr Ala
 595 600 605

Leu Tyr Arg Thr Leu Thr Ala Arg Gly Thr Gln Ala His Leu Val Leu
 610 615 620

Gly His Ser Val Gly Glu Ile Thr Ala Ala His Ile Ala Gly Val Leu
 625 630 635 640

Asp Leu Pro Asp Ala Ala Arg Leu Ile Thr Ala Arg Ala His Val Met
 645 650 655

Gly Gln Leu Pro His Gly Gly Ala Met Leu Ser Val Gln Ala Ala Glu
 660 665 670

His Asp Leu Asp Gln Leu Ala His Thr His Gly Val Glu Ile Ala Ala
 675 680 685

Val Asn Gly Pro Thr His Cys Val Leu Ser Gly Pro Arg Thr Ala Leu
 690 695 700

Glu Glu Thr Ala Gln His Leu Arg Glu Gln Asn Val Arg His Thr Trp
 705 710 715 720

Leu Lys Val Ser His Ala Phe His Ser Ala Leu Met Asp Pro Met Leu
 725 730 735

1993-1994-1995

Gly Ala Phe Arg Asp Thr Leu Asn Thr Leu Asn Tyr Gln Pro Pro Thr
 740 745 750

Ile Pro Leu Ile Ser Asn Leu Thr Gly Gln Ile Ala Asp Pro Asn His
 755 760 765

Leu Cys Thr Pro Asp Tyr Trp Ile Asp His Ala Arg His Thr Val Arg
 770 775 780

Phe Ala Asp Ala Val Gln Thr Ala His His Gln Gly Thr Thr Thr Tyr
 785 790 795 800

Leu Glu Ile Gly Pro His Pro Thr Leu Thr Thr Leu Leu His His Thr
 805 810 815

Leu Asp Asn Pro Thr Thr Ile Pro Thr Leu His Arg Glu Arg Pro Glu
 820 825 830

Pro Glu Thr Leu Thr Gln Ala Ile Ala Ala Val Gly Val Arg Thr Asp
 835 840 845

Gly Ile Asp Trp Ala Val Leu Cys Gly Ala Ser Arg Pro Arg Arg Val
 850 855 860

Glu Leu Pro Thr Tyr Ala Phe
 865 870

<210> 22

<211> 890

<212> PRT

<213> Streptomyces ambofaciens

<400> 22

Met Ser Gly Glu Leu Ala Ile Ser Arg Ser Asp Asp Arg Ser Asp Ala
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1980-1984-1985

Gly Tyr Val Arg Gly Glu Gly Gly Ala Ala Val Val Leu Lys Pro Leu
225 230 235 240

Ala Asp Ala Leu Ala Asp Gly Asp Pro Val Tyr Cys Val Val Arg Gly
245 250 255

Val Ala Val Gly Asn Asp Gly Gly Gly Pro Gly Leu Thr Ala Pro Asp
260 265 270

Arg Glu Gly Gln Glu Ala Val Leu Arg Ala Ala Cys Ala Gln Ala Arg
275 280 285

Val Asp Pro Ala Glu Val Arg Phe Val Glu Leu His Gly Thr Gly Thr
290 295 300

Pro Val Gly Asp Pro Val Glu Ala His Ala Leu Gly Ala Val His Gly
305 310 315 320

Ser Gly Arg Pro Ala Asp Asp Pro Leu Leu Val Gly Ser Val Lys Thr
325 330 335

Asn Ile Gly His Leu Glu Gly Ala Ala Gly Ile Ala Gly Leu Val Lys
340 345 350

Ala Ala Leu Cys Leu Arg Glu Arg Thr Leu Pro Gly Ser Leu Asn Phe
355 360 365

Ala Thr Pro Ser Pro Ala Ile Pro Leu Asp Gln Leu Arg Leu Lys Val
370 375 380

Gln Thr Ala Ala Ala Glu Leu Pro Leu Ala Pro Gly Gly Ala Pro Leu
385 390 395 400

Leu Ala Gly Val Ser Ser Phe Gly Ile Gly Gly Thr Asn Cys His Val
405 410 415

Val Leu Glu His Leu Pro Ser Arg Pro Thr Pro Ala Val Ser Val Ala
420 425 430

Ala Ser Leu Pro Asp Val Pro Pro Leu Leu Leu Ser Ala Arg Ser Glu
435 440 445

Gly Ala Leu Arg Ala Gln Ala Val Arg Leu Gly Glu Thr Val Glu Arg
450 455 460

Val Gly Ala Asp Pro Arg Asp Val Ala Tyr Ser Leu Ala Ser Thr Arg
465 470 475 480

Thr Leu Phe Glu His Arg Ala Val Val Pro Cys Gly Gly Arg Gly Glu
485 490 495

Leu Val Ala Ala Leu Gly Gly Phe Ala Ala Gly Arg Val Ser Gly Gly
500 505 510

Val Arg Ser Gly Arg Ala Val Pro Gly Gly Val Gly Val Leu Phe Thr
515 520 525

Gly Gln Gly Ala Gln Trp Val Gly Met Gly Arg Gly Leu Tyr Ala Gly
530 535 540

Gly Gly Val Phe Ala Glu Val Leu Asp Glu Val Leu Ser Met Val Gly
545 550 555 560

Glu Val Asp Gly Arg Ser Leu Arg Asp Val Met Phe Gly Asp Val Asp
565 570 575

Val Asp Ala Gly Ala Gly Ala Asp Ala Gly Ala Gly Ala Gly Ala Gly
580 585 590

Val Gly Ser Gly Ser Gly Ser Val Gly Gly Leu Leu Gly Arg Thr Glu
595 600 605

Phe Ala Gln Pro Ala Leu Phe Ala Leu Glu Val Ala Leu Phe Arg Ala
610 615 620

Leu Glu Ala Arg Gly Val Glu Val Ser Val Val Leu Gly His Ser Val
625 630 635 640

09720044-001301

Gly Glu Val Ala Ala Ala Thr Val Ala Gly Val Leu Ser Leu Gly Asp
645 650 655

Ala Val Arg Leu Val Val Ala Arg Gly Gly Leu Met Gly Gly Leu Pro
660 665 670

Val Gly Gly Gly Met Trp Ser Val Gly Ala Ser Glu Ser Val Val Arg
675 680 685

Gly Val Val Glu Gly Leu Gly Glu Trp Val Ser Val Ala Ala Val Asn
690 695 700

Gly Pro Arg Ser Val Val Leu Ser Gly Asp Val Gly Val Leu Glu Ser
705 710 715 720

Val Val Ala Ser Leu Met Gly Asp Gly Val Glu Tyr Arg Arg Leu Asp
725 730 735

Val Ser His Gly Phe His Ser Val Leu Met Glu Pro Val Leu Gly Glu
740 745 750

Phe Arg Gly Val Val Glu Ser Leu Glu Phe Gly Arg Val Arg Pro Gly
755 760 765

Val Val Val Val Ser Gly Val Ser Gly Gly Val Val Gly Ser Gly Glu
770 775 780

Leu Gly Asp Pro Gly Tyr Trp Val Arg His Ala Arg Glu Ala Val Arg
785 790 795 800

Phe Ala Asp Gly Val Gly Val Val Arg Gly Leu Gly Val Gly Thr Leu
805 810 815

Val Glu Val Gly Pro His Gly Val Leu Thr Gly Met Ala Gly Glu Cys
820 825 830

Leu Gly Ala Gly Asp Asp Val Val Val Val Pro Ala Met Arg Arg Gly
835 840 845

0970044.034
T3304.034

Arg Ala Glu Arg Glu Val Phe Glu Ala Ala Leu Ala Thr Val Phe Thr
 850 855 860

Arg Asp Ala Gly Leu Asp Ala Thr Ala Leu His Thr Gly Ser Thr Gly
 865 870 875 880

Arg Arg Ile Asp Leu Pro Thr Thr Pro Phe
 885 890

<210> 23

<211> 920

<212> PRT

<213> Streptomyces cinnamonensis

<400> 23

Met Ala Ala Ser Ala Ser Ala Ser Pro Ser Gly Pro Ser Ala Gly Pro
 1 5 10 15

Asp Pro Ile Ala Val Val Gly Met Ala Cys Arg Leu Pro Gly Ala Pro
 20 25 30

Asp Pro Asp Ala Phe Trp Arg Leu Leu Ser Glu Gly Arg Ser Ala Val
 35 40 45

Ser Thr Ala Pro Pro Glu Arg Arg Arg Ala Asp Ser Gly Leu His Gly
 50 55 60

Pro Gly Gly Tyr Leu Asp Arg Ile Asp Gly Phe Asp Ala Asp Phe Phe
 65 70 75 80

His Ile Ser Pro Arg Glu Ala Val Ala Met Asp Pro Gln Gln Arg Leu
 85 90 95

Leu Leu Glu Leu Ser Trp Glu Ala Leu Glu Asp Ala Gly Ile Arg Pro
 100 105 110

09720844-081301

Ala Thr Val Asp Glu Leu Thr Ala Ala Trp Arg Gly Arg Gly Arg Lys
740 745 750

Ala His His Leu Lys Val Ser His Ala Phe His Ser Pro His Met Asp
755 760 765

Pro Ile Leu Asp Glu Leu Arg Ala Val Ala Ala Gly Leu Thr Phe His
770 775 780

Glu Pro Val Ile Pro Val Val Ser Asn Val Thr Gly Glu Leu Val Thr
785 790 795 800

Ala Thr Ala Thr Gly Ser Gly Ala Gly Gln Ala Asp Pro Glu Tyr Trp
805 810 815

Ala Arg His Ala Arg Glu Pro Val Arg Phe Leu Ser Gly Val Arg Gly
820 825 830

Leu Cys Glu Arg Gly Val Thr Thr Phe Val Glu Leu Gly Pro Asp Ala
835 840 845

Pro Leu Ser Ala Met Ala Arg Asp Cys Phe Pro Ala Pro Ala Asp Arg
850 855 860

Ser Arg Pro Arg Pro Ala Ala Ile Ala Thr Cys Arg Arg Gly Arg Asp
865 870 875 880

Glu Val Ala Thr Phe Leu Arg Ser Leu Ala Gln Ala Tyr Val Arg Gly
885 890 895

Ala Asp Val Asp Phe Thr Arg Ala Tyr Gly Ala Thr Ala Thr Arg Arg
900 905 910

Phe Pro Leu Pro Thr Tyr Pro Phe
915 920

007004 001001

Leu Asp Ala Leu Arg Leu Arg Val His Thr Ala Tyr Gly Pro Trp Pro
385 390 395 400

Ser Pro Asp Arg Pro Leu Val Ala Gly Val Ser Ser Phe Gly Met Gly
405 410 415

Gly Thr Asn Cys His Val Val Leu Ser Glu Leu Arg Asn Ala Gly Gly
420 425 430

Asp Gly Ala Gly Lys Gly Pro Tyr Thr Gly Thr Glu Asp Arg Leu Gly
435 440 445

Ala Thr Glu Ala Glu Lys Arg Pro Asp Pro Ala Thr Gly Asn Gly Pro
450 455 460

Asp Pro Ala Gln Asp Thr His Arg Tyr Pro Ala Leu Ile Leu Ser Ala
465 470 475 480

Arg Ser Asp Ala Ala Leu Arg Ala Gln Ala Glu Arg Leu Arg His His
485 490 495

Leu Glu His Ser Pro Gly Gln Arg Leu Arg Asp Thr Ala Tyr Ser Leu
500 505 510

Ala Thr Arg Arg Gln Val Phe Glu Arg His Ala Val Val Thr Gly His
515 520 525

Asp Arg Glu Asp Leu Leu Asn Gly Leu Arg Asp Leu Glu Asn Gly Leu
530 535 540

Pro Ala Pro Gln Val Leu Leu Gly Arg Thr Pro Thr Pro Glu Pro Gly
545 550 555 560

Gly Leu Ala Phe Leu Phe Ser Gly Gln Gly Ser Gln Gln Pro Gly Met
565 570 575

Gly Lys Arg Leu His Gln Val Phe Pro Gly Phe Arg Asp Ala Leu Asp
580 585 590

0970044-001301

Gly Ala Pro Val Asp Asp Arg Thr Met Ala Thr Pro Ala Tyr Trp Val
 805 810 815

Arg His Val Arg Glu Ala Val Arg Phe Gly Asp Gly Ile Arg Ala Leu
 820 825 830

Gly Lys Leu Gly Thr Gly Ser Phe Leu Glu Val Gly Pro Asp Gly Val
 835 840 845

Leu Thr Ala Met Ala Arg Ala Cys Val Thr Ala Ala Pro Glu Pro Gly
 850 855 860

His Arg Gly Glu Gln Gly Ala Asp Ala Asp Ala His Thr Ala Leu Leu
 865 870 875 880

Leu Pro Ala Leu Arg Arg Gly Arg Asp Glu Ala Arg Ser Leu Thr Glu
 885 890 895

Ala Val Ala Arg Leu His Leu His Gly Val Pro Met Asp Trp Thr Ser
 900 905 910

Val Leu Gly Gly Asp Val Ser Arg Val Pro Leu Pro Thr Tyr Ala Phe
 915 920 925

<210> 25

<211> 922

<212> PRT

<213> *Streptomyces fradiae*

<400> 25

Met Ser Ser Ala Leu Arg Arg Ala Val Gln Ser Asn Cys Gly Tyr Gly
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0920344.0302.60

Thr Glu Glu Ser Thr Thr Val Met Glu Arg Met Gly Ala Leu Ser Pro
225 230 235 240

Asp Gly Arg Cys His Thr Phe Asp Ala Arg Ala Asn Gly Tyr Val Arg
245 250 255

Gly Glu Gly Gly Gly Ala Val Val Leu Lys Pro Leu Asp Ala Ala Leu
260 265 270

Ala Asp Gly Asp Arg Val Tyr Cys Val Ile Lys Gly Gly Ala Val Asn
275 280 285

Asn Asp Gly Gly Gly Ala Ser Leu Thr Thr Pro Asp Arg Glu Ala Gln
290 295 300

Glu Ala Val Leu Arg Gln Ala Tyr Arg Arg Ala Gly Val Ser Thr Gly
305 310 315 320

Ala Val Arg Tyr Val Glu Leu His Gly Thr Gly Thr Arg Ala Gly Asp
325 330 335

Pro Val Glu Ala Ala Ala Leu Gly Ala Val Leu Gly Ala Gly Ala Asp
340 345 350

Ser Gly Arg Ser Thr Pro Leu Ala Val Gly Ser Val Lys Thr Asn Val
355 360 365

Gly His Leu Glu Gly Ala Ala Gly Ile Val Gly Leu Ile Lys Ala Thr
370 375 380

Leu Cys Val Arg Lys Gly Glu Leu Val Pro Ser Leu Asn Phe Ser Thr
385 390 395 400

Pro Asn Pro Asp Ile Pro Leu Asp Asp Leu Arg Leu Arg Val Gln Thr
405 410 415

Glu Arg Gln Glu Trp Asn Glu Glu Asp Asp Arg Pro Arg Val Ala Gly
420 425 430

Glu Arg Val Asp Val Val Gln Pro Val Thr Trp Ala Val Met Val Ser
645 650 655

Leu Ala Arg Tyr Trp Gln Ala Met Gly Val Asp Val Ala Ala Val Val
660 665 670

Gly His Ser Gln Gly Glu Ile Ala Ala Ala Thr Val Ala Gly Ala Leu
675 680 685

Ser Leu Glu Asp Ala Ala Ala Val Val Ala Leu Arg Ala Gly Leu Ile
690 695 700

Gly Arg Tyr Leu Ala Gly Arg Gly Ala Met Ala Ala Val Pro Leu Pro
705 710 715 720

Ala Gly Glu Val Glu Ala Gly Leu Ala Lys Trp Pro Gly Val Glu Val
725 730 735

Ala Ala Val Asn Gly Pro Ala Ser Thr Val Val Ser Gly Asp Arg Arg
740 745 750

Ala Val Ala Gly Tyr Val Ala Val Cys Gln Ala Glu Gly Val Gln Ala
755 760 765

Arg Leu Ile Pro Val Asp Tyr Ala Ser His Ser Arg His Val Glu Asp
770 775 780

Leu Lys Gly Glu Leu Glu Arg Val Leu Ser Gly Ile Arg Pro Arg Ser
785 790 795 800

Pro Arg Val Pro Val Cys Ser Thr Val Ala Gly Glu Gln Pro Gly Glu
805 810 815

Pro Val Phe Asp Ala Gly Tyr Trp Phe Arg Asn Leu Arg Asn Arg Val
820 825 830

Glu Phe Ser Ala Val Val Gly Gly Leu Leu Glu Glu Gly His Arg Arg
835 840 845

09700041.004304

<210> 27
 <211> 118
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 Oligonucleotide

<400> 27
 aaccgggtccc tcaggctcga tgcattctccc tcgtcaogag gactagtgcg gtccgggtgc 60
 agccagaacc gcttgccctg gaaaggatac gtaggcagat ctaccagtcc ggcccggc 118

<210> 28
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 Oligonucleotide

<400> 28
 ccatatggcc gcatccgcgt cagcgt 26

<210> 29
 <211> 31
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 Oligonucleotide

<400> 29
 ggctagcggg tcctcgtccg tgccgaggtc a 31

<210> 30

<211> 48

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide

<400> 30

aattcacatc accatcacca tcactagtag gaggtctggc catctaga

48

<210> 31

<211> 48

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide

<400> 31

agcttctaga tggccagacc tcctactagt gatggtgatg gtgatgtg

48

<210> 32

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide

<400> 32

tggaccgccg ccaattgcct aggcgggccg aacccggct

39

1434460

<210> 33
 <211> 36
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 Oligonucleotide

<400> 33
 cctgcaggcc atcgcgacga ccgcgaccgg ttcgcc 36

<210> 34
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 Oligonucleotide

<400> 34
 ccacatatgc atgtccccgg cgaggaa 27

<210> 35
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 Oligonucleotide

<400> 35
 ccctgtccgg agaagaggaa ggcgaggccg 30

<210> 36

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide

<400> 36

ccatatgtct ggagaactcg cgatttcccg cagt

34

<210> 37

<211> 28

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:

Oligonucleotide

<400> 37

ggctagcggg tcgtcgtcgt cccggctg

28

<210> 38

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide

<400> 38

tacctaggcc gggccggact ggtcgacctg ccgggtt

37

09760344-094304

<210> 39

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
Oligonucleotide

<400> 39

atgttaaccg gtcgcgagcgtctctccgtct

30

<210> 40

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
Oligonucleotide

<400> 40

atgttaacgg gtctgccggtg gccgagcgg ac

32

<210> 41

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
Oligonucleotide

<400> 41

cttctagact atgaattccc tccgccagc

30

TOE T90 1 490260

<210> 42

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide

<400> 42

taagatcttc cgacgtacgc gttccagc

28

<210> 43

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide

<400> 43

atgctagcca ctgcgccgac gaatcaccgg tgg

33

<210> 44

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide

<400> 44

tacctgaggg accggctagc gggctgcccg cgtg

34

<210> 45
 <211> 34
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 Oligonucleotide

<400> 45
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34

<210> 46
 <211> 28
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 Oligonucleotide

<400> 46
 cgttcctgag gtcgctggcc caggcgta

28

<210> 47
 <211> 28
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 Oligonucleotide

<400> 47
 cgaagcttga caccgcggcg cggcgcgg

28

69344-034
 TCEB0-T49265

<210> 48

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
Oligonucleotide

<400> 48

gcgcgccaat tgcgtgcaca tctcgat

27

<210> 49

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
Oligonucleotide

<400> 49

cctgcaggcc atcgcgacga ccgcgaccgg ttcgccg

37

<210> 50

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
Oligonucleotide

<400> 50

gtctcaagct tcggcatcag cggcaccaa

29

<210> 51

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide

<400> 51

cgtgcgatat ccctgctcgg cgagcgca

28

<210> 52

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide

<400> 52

gatggcctgc aggctgcccg gcggtgtgag ca

32

<210> 53

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide

<400> 53

gccgaagctt gagacccccg cccggcgcgg tcgc

34